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Transmission of bovine tuberculosis (Mycobacterium bovis) from badgers (Meles meles) to cattle.

TRANSMISSION OF BOVINE TUBERCULOSIS (*MYCOBACTERIUM BOVIS*) FROM BADGERS (*MELES MELES*) TO CATTLE

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ABSTRACT

In this thesis, seasonal patterns of spatial behaviour, dunging and urinatory behaviour and anal gland secretion for a badger (*Meles meles*) population are quantified. The role of these excretory products and secretions in territoriality and the significance of urine and faeces in the transmission of bovine tuberculosis (*Mycobacterium bovis*) from badgers to cattle is assessed.

To look at the patterns of scent marking and spatial behaviour of individuals of known age and sex, spool-and-line tracking was used to follow selected animals, which had also been injected subcutaneously with fluorescein dye to monitor their pattern of urination, defecation and anal gland deposition.

Forty two badgers from five contiguous social groups on the Cotswold escarpment in Gloucestershire were spool-and-line tracked between June 1991 and May 1992. For these five social groups mean group size in 1991 was 17.0 $\pm$ 2.8 badgers and mean territory size 37.2 $\pm$ 7.1 ha. All five groups had adjacent groups of badgers on all sides.

Contrary to earlier reports, there was no seasonal pattern of faecal deposition by individuals, and seasonal variations in the number of faeces in latrines probably reflect variations in faecal decomposition rates. Instead, urine appeared to be a more important territorial mark. Territorial marking and boundary patrolling by adult males in particular was most pronounced in the summer and autumn, and not during the main mating season. Whilst all age and sex categories played a role in territorial marking, adult females visited more latrines in the spring than any other season, with latrine visits declining in the summer and autumn. Whilst not all hypotheses on badger social behaviour were supported by the field data, the evidence favoured the defence of food resources and not monopolising access to oestrous sows as the main factor leading to the evolution of group living and territorial behaviour in badgers.

Badgers are believed to be responsible for a high proportion of the cases of bovine tuberculosis in cattle in south-west England, where in certain limited areas comparatively high numbers of cattle continue to fail the tuberculin test. Environmental contamination with urine from infectious badgers is probably the main mode of transmission. Badgers may urinate on pasture after crossing through a linear feature, and the number of these crossing point urinations increases with the number of linear feature crossed. These urinations were not at latrines, and had a peak occurrence between March and May, corresponding to a period when cattle are believed to become infected. Since high risk areas have been found to be characterised by a greater degree of habitat heterogeneity and more linear features, it is hypothesised that these crossing point urinations probably pose a significant risk to cattle, and areas with increased numbers of linear features have greater levels of contamination of pasture with badger urine and hence greater chance of disease transmission.

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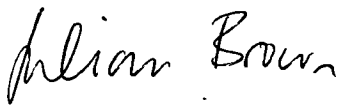
DEDICATION

To my parents

DECLARATION

Bait marking data for Staffordshire, Avon, Cornwall and Gloucestershire and social group sizes for Gloucestershire were supplied by the Ministry of Agriculture, Fisheries and Food. Bite wounding data were supplied by Warren Cresswell and Stephen Harris. With these exceptions, I declare that the work contained in this thesis is my own and has not been submitted for any other degree or award.



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CHAPTER 1

GENERAL INTRODUCTION

1.1 Eradication of tuberculosis in cattle

At the beginning of the century bovine tuberculosis (*Mycobacterium bovis*) was one of the most serious diseases of cattle in Great Britain. By 1934, at least 40% of dairy cattle were infected with tuberculosis and in 1935 the first voluntary national eradication programme for the disease was implemented. This eradication programme however was halted due to the war (1939-1945). In 1950 the current compulsory eradication campaign was introduced on an area by area basis. For this an area was declared attested after all the herds had been tested twice and any positive cattle had been removed for slaughter. By 1960 all areas of Great Britain had been declared attested and at this stage the incidence of reactor herds was about one herd in fifty. Over the next ten years the incidence of tuberculosis in cattle continued to fall, except in parts of south-west England where levels remained static. Between 1970-1972 a team of veterinarians from the Ministry of Agriculture, Fisheries and Food investigated these higher levels of tuberculosis in cattle in the south-west of England and concluded that several factors may be responsible. One of their recommendations was that wildlife be examined as they may be acting as a reservoir of infection. The discovery of bovine tuberculosis in a dead badger (*Meles meles*) on a Gloucestershire farm in 1971, together with the recommendation to examine the possibility of wildlife acting as a reservoir of *M.bovis* for cattle, led to an investigation to measure the prevalence of tuberculosis in badgers in the south-west of England. These investigations led the Ministry of Agriculture, Fisheries and Food to conclude in 1973 that badgers were a reservoir

of infection and that action was required against infected badgers that posed a threat to cattle.

Badger control commenced in August 1975, when the Ministry of Agriculture, Fisheries and Food introduced a policy of gassing setts with hydrogen cyanide gas in areas where badgers were believed to be responsible for transmission of bovine tuberculosis to cattle (Muirhead, Gallagher & Burn 1974; Gallagher, Muirhead & Burn 1976). The Consultative Panel on Badgers and Bovine Tuberculosis was set up to discuss the problems associated with bovine tuberculosis in the badger population, which met for the first time in September 1975. In 1976 the Ministry of Agriculture, Fisheries and Food initiated a long-term research programme at a site on the Cotswold escarpment in Gloucestershire to investigate the population biology of badgers and the epidemiology of tuberculosis in the badger population.

Lord Zuckerman was asked in September 1979 by the then Minister of Agriculture, Peter Walker, to look at the policy of badger control and advise on how future operations should be tackled. During the period of policy review (September 1979-October 1980) the gassing of new setts was suspended and restricted only to those areas already cleared of the disease. Lord Zuckerman recommended a continuation of the existing policy, although he also suggested that the efficiency of hydrogen cyanide gas in setts be examined. Experiments conducted by the Chemical Defence Establishment revealed that the gas was present in setts at concentrations considerably lower than required to humanely kill badgers. As a result in July 1982 the Minister of Agriculture announced the abolishment of gassing as the method of control and the introduction of live trapping followed by humane killing. This policy involved trapping in a centrifugal manner until all infected groups surrounding the point of infection were removed. Lord Zuckerman also recommended that three years after his own report was published another policy review should be conducted. This second review was undertaken by a three

man team and in March 1986 a second report on the policy of badger control was published (Dunnet, Jones & McInerney 1986). As with the earlier report it also endorsed a continuation of badger control, although it did recommend a number of changes. One of these changes was the need to develop a diagnostic test for live badgers. This would involve detecting those badgers producing antibodies against *M. bovis* which are assumed to have been exposed to the disease. Badgers reacting positively to the test would be destroyed, while those badgers not reacting would be released. Thus this test would serve as a more discriminatory and therefore more acceptable method of badger control. The present policy of only trapping on 'infected land', i.e. where transmission is thought to have taken place is also intended to serve as a more discriminatory method of badger control.

The report also highlighted the need for a policy to limit the transmission of disease from badgers to cattle by dealing with identifiable and avoidable risks. Unfortunately there are still few quantified data indicating how *M.bovis* might be transmitted nor how high risk situations might be identified.

1.2 Bovine tuberculosis and badgers

The role of the badger as a reservoir of bovine tuberculosis in the south-west of England was revealed in the early 1970s when up to 20% of badgers examined were found to be infected (Muirhead, Gallagher & Burn 1974; Gallagher, Muirhead & Burn 1976). The levels of *M. bovis* infection in cattle in the south-west have remained persistantly higher than the rest of Great Britain (Zuckerman 1980; Wilesmith 1983) with the number of herd breakdowns rising to 104 in the south-west in 1990, compared to only 39 herd breakdowns in 1990 in the rest of Britain (Ministry of Agriculture, Fisheries and Food 1991).

Since the discovery of a tuberculous badger on a Gloucestershire farm, circumstantial evidence has accumulated linking badgers with the transmission of

bovine tuberculosis in the south-west of England (Muirhead, Gallagher & Burn 1974; Gallagher, Muirhead & Burn 1976; Ministry of Agriculture, Fisheries and Food 1976, 1977, 1979). This evidence includes the finding that *M. bovis* from infected badgers produces tuberculous lesions when inoculated into cattle (Little, Burn & Stuart 1975), while healthy calves can become infected when in contact with either artificially or naturally infected badgers (Little, Naylor & Wilesmith 1982). Also a close relationship has been found between the incidence of tuberculosis in badgers and herd breakdowns (Muirhead, Gallagher & Burn 1974).

The route by which bovine tuberculosis might spread from badgers to cattle has been little studied and remains speculative, although it is believed that cattle may pick up infection while grazing pasture contaminated by an infected badger (Muirhead, Gallagher & Burn 1974; Ministry of Agriculture, Fisheries and Food 1979). This could result from contamination of pasture by infected badger urine, sputum and/or faeces (Muirhead, Gallagher & Burn 1974). Another source of environmental contamination might be pus from infected bite wounds, since *M. bovis* has been recovered from the exudate of wounds in live badgers (Muirhead, Gallagher & Burn 1974). Although badgers and cattle are generally thought to avoid each other (Benham & Broom 1989), behavioural changes in badgers in an advanced stage of infection have been reported; such badgers may lose their fear of man and inhabit farm buildings (Muirhead, Gallagher & Burn 1974; Cheeseman & Mallinson 1981). Such behaviour may increase the risk of transmission between badgers and cattle, especially if cattle food is contaminated. Contamination around the sett may also be of significance, since cattle have been observed to rub their heads in the exposed earth around setts and then lick the ground and each other's faces (Benham 1984).

Of these various possibilities, badger faeces are likely to be of minor importance in the transmission of *M. bovis* from badgers to cattle because they contain a relatively low number of bacilli, and these are only exposed in small

numbers by weathering (Ministry of Agriculture, Fisheries and Food 1976, 1979). Furthermore, faeces are generally deposited in pits in discrete areas (latrines) and a relatively large proportion of these are inaccessible to cattle; cattle also strongly avoid the ingestion of pasture contaminated with faeces (Benham & Broom 1991). Conversely, up to 300,000 *M. bovis* ml^{-1} of urine have been recovered from infected badgers (Gallagher & Horwill 1977); with a badger excreting on average about 30 ml of urine at a time (Ministry of Agriculture, Fisheries and Food 1976), this would result in the emission of a considerable number of bacilli. Whilst the pattern of urine deposition by badgers has been poorly documented, it is known that cattle perform more sniffing in response to urine than faeces (Benham & Broom 1991). Therefore contaminated badger urine would appear to pose a greater risk than faeces for transmission.

1.3 Scent marking in carnivores

One of the major problems associated with studying scent marking behaviour in carnivores is the difficulty in detecting certain types of scent marks, in particular urine and many of the glandular secretions. As a result, much of the information relating to scent marking in carnivores comes from species inhabiting cold regions where snow cover retains signs of scent marks e.g. wolves (*Canis lupus*) (Peters & Mech 1975; Rothman & Mech 1979), wolverines (*Gulo gulo*) (Pullianen & Ovaskainen 1975) and coyotes (*Canis latrans*) (Barrette & Messier 1980; Bowen & McTaggart Cowan 1980).

Mammalian scent marking has been broadly reviewed by Ralls (1971), Eisenberg & Kleiman (1972) and Johnson (1973). Gorman (1980) has reviewed the role of glandular secretions in scent marking, while Macdonald (1980) has reviewed the pattern of scent marking with urine and faeces amongst carnivore communities. Kleiman (1966) reviewed scent marking in the Canidae and clearly differentiated

scent marking from simple elimination by the following criteria: (a) excreta was orientated to specific objects, (b) elicited by familiar landmarks and novel stimuli and (c) repeated frequently on the same objects. The importance of distinguishing between scent marking and elimination has also been discussed by Barrette (1977), although Barrette & Messier (1980) considered all urine signs to be potential scent marks, while Dunbar (1978) and Bekoff (1979) suggested that all excretions may act as potential marks to recipients irrespective of the intent of the producer. Certainly there is no evidence to suggest any chemical variation between excreta used for marking and that produced simply for elimination. This may explain the excretory behaviour of itinerant wolves, observed in both sexes. To avoid or at least reduce the chances of their excreta from being detected by resident pack members, Rothman & Mech (1979) found no scratching associated with urination by itinerants and that urine and faeces were deposited away from travel routes. Itinerants also neither overmarked alien scent, nor marked in the vicinity of kills, both of which were marked heavily by resident pack members. Thus by concealing their excreta, itinerant wolves are able to maintain a low profile whilst in the territories of resident wolf packs. Wells & Bekoff's (1981) criteria for marking associated with the act of urination would be an associated behaviour, such as scratching after urination or urinating over previous spots of urine. Another important criterion of scent marking is that less urine is generally expelled during marking than elimination (Peters & Mech 1975; Barrette 1977; Henry 1977; Bekoff 1979; Bell 1980; Macdonald 1980). Macdonald (1979) found that a tame fox (*Vulpes vulpes*) leash-walked along paths and field borders in midwinter produced over 100 token urinations per hour.

Amongst the Carnivora, faeces and urine are excreted at a wide variety of sites. The pattern and frequency may vary with sex, social and reproductive status. It is probable that many different messages are conveyed by scent marks, although in most cases the exact function of the messages is unknown. As well as inter-

specific variation in scent marking behaviour, some species show considerable intra-specific variation in the pattern of marking, a feature largely dependent on habitat.

Field studies on carnivores have revealed that carnivores often concentrate scent marks on or close to territory boundaries, e.g. in otter (*Lutra lutra*) (Erlinge 1968), spotted hyaena (*Crocuta crocuta*) (Kruuk 1972), wolf (Peters & Mech 1975), badger (Kruuk 1978; Cheeseman *et al.* 1981; Roper, Shepherdson & Davies 1986; Pigozzi 1990), stoat (*Mustela erminea*) (Erlinge, Sandell & Brinck 1982), coyote (Barrette & Messier 1980; Bowen & McTaggart Cowan 1980), demonstrating the importance of scent marking in the maintenance of territories. However, scent marks are often deposited within the territory, at junctions of paths e.g. badger (Kruuk, Gorman & Leitch 1984), wolf (Peters & Mech 1975), or on prominent objects e.g. red fox (Burrows 1968; Henry 1977; Macdonald 1979) and wolf (Peters & Mech 1975).

As scent marks play a role in communication between conspecifics, they should be distributed at localities that would maximise their detection by other animals. This can be seen in the above examples with animals depositing excreta at trail junctions and also on prominent objects. Peters & Mech (1975) found that wolves would deposit urine on elevated locations such as on trees, shrubs, rocks and snow banks. Depositing urine well above ground facilitated the dispersal of odour by wind, increased the evaporative surface area of the urine as it trickled down the elevated surface, and also minimised the chance of the urine from being covered by snow or washed away by rain.

Faeces have been more widely studied as scent marks due to the ease of detecting faeces compared to urine. Foxes generally deposit faeces singly, and as with urine, almost always deposit faeces on or near visually conspicuous objects. The gray fox (*Urocyon cinereoargenteus*) and cacomistle (*Bassariscus astutus*) deposit faeces in a similar pattern to the red fox, although they occasionally deposit faeces in large quantities at particular sites (Trapp 1978). Other species consistently

deposit faeces at particular sites (latrines). These include spotted hyaena (Kruuk 1972), palm civet (*Paradoxurus hermaphroditus*) (Bartels 1964), pine marten (*Martes martes*) (Lockie 1966), dwarf mongoose (*Helogale undulata*) (Rasa 1977), long nosed mongoose (*Crossarchus alexandri*) (Kingdon 1977), African polecat (*Poecilogale albinucha*) (Alexander & Ewer 1959) and raccoon dog (*Nyctereutes procyonoides*) (Ikeda, Eguchi & Ono 1979). Some species that leave large numbers of faeces at latrines also defecate away from latrines. These faeces are generally deposited singly and may either have no communicative function or convey different information to those located at latrines. This behaviour has been observed for a population of golden jackals (*Canis aureus*) (Macdonald 1979), where single faeces deposited away from latrines were frequently deposited on conspicuous objects, such as bushes. Golden jackal latrines were distributed along territory boundaries, whereas single faeces were deposited within territories. In contrast badgers have both boundary latrines and latrines within territories (hinterland latrines). Kruuk (1978) found that badger boundary latrines were linked by paths and that boundary latrines contained more faeces than hinterland latrines. Both types of latrine were located more frequently by conspicuous landmarks than predicted by chance. In some species hinterland latrines were often associated with trails and trail junctions and were even located in close proximity to lairs. Given the value of a lair as a resource and the activity centred around lairs, they are ideal for the transfer of information between group members and/or intruders. This behaviour of concentrating latrines around a lair can be seen in otters inhabiting coastal areas but not by otters maintaining inland territories. Kruuk & Hewson (1978) suggested that this intra-specific difference in otter scent marking behaviour is due to ecological reasons. Inland otter territories could be entered along well defined routes, and so latrines would be most effective along the territory boundary. However, for coastal otters, where territories could be entered from anywhere along the seafront, latrines were most effective around holts.

European badgers, spotted hyaenas, golden jackals and otters living in riverine habitats are the only carnivores that have been found to use latrines for boundary marking. Macdonald (1980) suggested that this behaviour was associated with group living species in high density populations which defend relatively small areas. They are also long lived species whose borders are stable over periods of time. Macdonald (1980) also suggested that the distribution of latrines may reflect the quality and distribution of resources. For example, where food patches are stable over time, then this may result in heavy marking of the territory, particularly in the vicinity of the boundary. Where resources are clumped, then scent marks would be more concentrated at these sites. This is the case in spotted hyaenas (Bearder & Randall 1978), which establish temporary latrines in the vicinity of large kills. Where resources are ephemeral scent marks may be inappropriate for resource defence. Thus the pattern of resource distribution and its affect on the social organisation of a particular species may be the principal factor regulating the distribution of scent marks.

Carnivores produce a wide variety of odours which probably serve numerous functions. Spotted hyaenas (Kruuk 1972) and badgers (Kruuk 1978) scrape at latrines, a behaviour also observed in wolves and coyotes after urinating (Peters & Mech 1975; Bekoff & Diamond 1976). This behaviour is probably associated with the deposition of odour from the interdigital glands. Spotted hyaenas (Kruuk 1972) leave anal gland pastings at latrines, while civets (*Civettictis civetta*) deposit perineal gland secretions at civetries (Randall 1977). Badgers leave anal and subcaudal gland secretions at latrines and also deposit subcaudal gland secretion on bedding material within setts and on other members of the social group, especially females and cubs (Kruuk, Gorman & Leitch 1984). These authors found that badgers were able to distinguish individuals from their subcaudal gland secretion. Latrines therefore contain a wide variety of scent marks, and as well as each mark producing a different odour, combinations of marks may be interpreted in a different manner.

The decay of scent marks may also ensure that a particular odour is most effective during a limited period after its deposition. Apart from the information available from the scent marks themselves, the overall pattern of deposition may have communicative significance.

The pattern of scent marking in the badger is clear at the group level, but the role of individuals within the group is unknown. Also, seasonal patterns of faecal production in the badger at the group level have been studied, yet the use of urine as a scent marker and the ecological significance of urine for disease transmission in the badger have not previously been recorded. The primary objective of the thesis was to investigate the possible mode of transmission of bovine tuberculosis from badgers to cattle and in particular the role of excreta in the transmission process. This was studied at an area on the Cotswold escarpment in Gloucestershire. This study site was ideal given the high density of badgers, together with the high prevalence of bovine tuberculosis in the badger population. To understand better the risk of TB transmission posed by individual badgers required the development of techniques to record the detailed movement patterns of badgers and the unique opportunity to gather scent marking data from individual animals to establish sites of excreta deposition and seasonal patterns of excretory behaviour. The techniques used enabled the role of the individual in territoriality to be analysed and the possible reasons behind the evolution of group living in the badger.

1.4 Study area and animals

The study site covered an area of approximately 9 km^2 of hilly terrain, ranging from 47-237m above sea level (Cheeseman *et al.* 1988). Deciduous woodland on the steep sided valleys occupied around 20% of the area, with scattered built up areas another 20% and permanent pasture and arable land 45% and 15% respectively. The farming in the region was mixed, with beef and dairy

cattle occurring largely on the hilly areas. This area supported a total of 34 social groups with approximately 20 adults km^{-2} (Cheeseman *et al.* 1981; 1987), with a mean nearest neighbour distance between main setts of 325m (Cheeseman *et al* 1985).

Badgers were trapped routinely by the Ministry of Agriculture, Fisheries and Food as part of a long term study of the epidemiology of *M. bovis* in the badger population. As part of the trapping operation, samples such as faeces, urine, tracheal aspirate and blood for serology were routinely collected. Where appropriate swabs were taken from open bite wounds and ruptured lymph node abscesses, while enlarged lymph nodes were aspirated with a hypodermic syringe. Both cultural and biological techniques were used to isolate *M. bovis*. The weight and rectal temperature of each badger was also recorded. Animals were aged and sexed and marked with a combination of ear tags and tattoo (Cheeseman & Harris 1982). Ear tags were applied to both ears and the tattoo placed on the ventral abdomen in the inguinal region. Animals were held in captivity to prevent re-capture during the catching period which continued for only 48 hours to prevent stress and possible disruption of social groups.

For the present study, five contiguous groups in the south-west corner of the area were selected; all five had adjacent groups of badgers on all sides. These particular groups were located within an area with a history of herd breakdowns. Bait marking was used to obtain information on territory sizes for the five study groups (Kruuk 1978, Cheeseman *et al.* 1981). Bait consisted of peanuts, syrup and plastic pellets (Alcathene supplied by I.C.I. Plastics Division, Bessemer Road, Welwyn Garden City, Herts.). Different coloured plastic pellets were placed at each of the main setts over a two-week period in February 1991 and 1992. The study area was surveyed after this period and latrines inspected for the presence of plastic pellets. By relating latrines containing pellets to the sett from which they originated it was possible to map out those latrines visited by members of each

social group. Knowledge of the location of the boundary path that connected the peripheral latrines of a group, combined with the bait marking, enabled latrines to be differentiated into boundary and hinterland latrines. For these five study groups mean territory size in 1991 was 37.2 $\pm$ 7.1 ha, mean territorial boundary length 2.71 $\pm$ 0.23 km, mean group size 17.0 $\pm$ 2.8 badgers, and mean number of setts per social group (excluding the main sett) 5.0 $\pm$ 0.6. Within these territories mean area of woodland was 6.24 $\pm$ 2.38 ha and mean area of permanent pasture 25.36 $\pm$ 2.86 ha.

1.5 The thesis

1.5.1 Thesis structure

Following the introduction, Chapter 2 describes how spool-and-line tracking in conjunction with a biomarker was used to follow the detailed movement patterns of individual animals and together enabled badger excretory products to be located. Construction of the spool-and-line and the problems associated with spool-and-line tracking such as tracking animals the night after capture are included, together with the protocol used to ensure the minimum impact on the animals behaviour the night following capture. Chapter 3 examines how these techniques were implemented to analyse the role of the individual within the social group in scent marking and territorial behaviour. Scent marking and spatial data from different age and sex categories were used to compare the food-based and sex-based hypotheses which attempt to explain the evolution of group living in the badger. Chapter 4 examines the significance of scent marking by badgers in the transmission of bovine tuberculosis from badgers to cattle. Data were collected using the combination of spool-and-line tracking and biomarker and were analysed at the group rather than the individual level. Analyses included the seasonal pattern of excretory behaviour

in different habitats and the identification of the different sites used by the social group for the deposition of excreta. The possible stimulus for urination and defecation at latrines was also examined. Chapter 5 combines spool-and-line data with data collected from latrines visited by focal badgers. This chapter examines the significance of the seasonal pattern of latrine activity in badger territoriality and TB transmission. Chapter 6 examines which parameters are important factors in controlling the density of latrines on the study site and uses stepwise multiple regression analysis to develop a simple predictive model for estimating the density of latrines in different areas. Chapter 7 provides a summary discussion of the main findings of the work.

1.5.2 Statistical analysis

The statistical tests in this thesis include one- and two-way analysis of variance with blocks where appropriate, Friedman's analysis of variance, Kruskal-Wallis one-way analysis of variance, matched sample t-tests, stepwise multiple regression analysis, linear regression analysis and X^2. Analysis of variance and matched sample t-tests are both robust parametric tests whose validity is only slightly affected by data showing considerable departures from the assumptions of normality and homoscedasticity of variances (Zar 1984; Sokal & Rohlf 1987). For seasonal analyses, data were grouped as follows: spring March-May, summer June-August, autumn September-November, and winter December-February. Three different badger age categories were recognised; young of the year (cubs), yearlings and animals $\geq$ 3 years (adults). For some analyses, adult males were divided into young (those in their third year) and old animals ($\geq$ 4 years). Animals entered the next age class at the start of the spring season. Although radio tracking would have resulted in larger sample sizes, because precise movement patterns of individual animals were required, the spool-and-line technique meant that sample sizes were of

necessity small. This posed particular problems when analysing seasonal variations, especially in Chapter 3 where individual age and sex categories were also included in the analyses. In Chapter 3 the most obvious analysis would have been three-way analysis of variance with sex, age and season as variables. This however was not possible as some blocks contained no data. As a result one-way and two-way analysis of variance statistical tests were used. In Table 3.1 for example when analysing differences in the number of boundary and hinterland urinations between different age and sex categories within seasons, one-way analysis of variance was used. In such analyses, each data point was an individual animal and hence was independent. When analysing seasonal patterns in urinatory behaviour for particular age and sex categories two-way analysis of variance was used. This was because some animals in this analysis were followed in more than one season. When measurements are taken from the same individual the measurements tend to be correlated with each other. This correlation is taken into account by treating individuals as blocks in two-way analysis of variance (SAS Institute Inc.; A. Dennis pers. comm.). Also in this chapter, since the data on excretion rates, boundary and hinterland latrine visits were small and some of the observations were zero, they were subjected to square root transformation to improve the normality of the data before analysis of variance (Bartlett 1936). Proportion and percentage data were arcsine transformed before analysis of variance.

Several procedures are available to assess departures from normality although these procedures have relatively unsatisfactory performance in testing for these departures (Zar 1984). In this study the distribution of the data were examined graphically from plots of frequency distribution and not by statistical procedures. When data showed extreme departures from the assumptions of normality non-parametric tests were used. Due to the robustness of the analysis of variance test and that this test is only slightly affected by data showing considerable departures from normality (Zar 1984; Sokal & Rohlf 1987), together with the

unreliability of tests for normality, it was not considered that the method of selecting particular statistical tests in this thesis was inappropriate.

In Chapters 3 and 4, for Kruskal-Wallis analysis of variance, where a particular badger was tracked in more than one season a single data point was selected at random for that badger. Likewise in Chapter 5, when comparing between seasons, data from a particular latrine occurring in more than one season were randomly removed to leave just one data point for that latrine throughout the year. This procedure was not performed, however, when latrines were treated as blocks in analysis of variance statistical tests. Within seasons, data obtained on more than one occasion for a particular latrine were averaged. For analyses in Chapter 5, latrines were either grouped according to habitat (pasture, woodland and arable), or latrine type, i.e. boundary and hinterland latrines. Boundary latrines were those latrines situated on the boundary run, hinterland latrines occurred elsewhere within a group's territory. Due to a small sample size for arable latrines, this category was omitted from many of the analyses, although data from these latrines were incorporated into the overall analyses of latrine use. The chi-square analysis for proportions (Zar 1984) in Chapter 5 used independent proportions.

Stepwise multiple regression techniques were applied to the data in Chapter 6 to produce the predictive model, using Minitab, a general purpose statistical system (Ryan, Joiner & Ryan 1985). Stepwise multiple regression analyses employed a backward elimination of independent variables whose t values were less than the critical t value (Zar 1984). Simple linear regressions were performed to examine how individual parameters influenced latrine density. The F-statistic and R^2 (the percentage explained variation) are quoted for all multiple and linear regressions (Chatfield & Collins 1980). In order to provide a control on the reliability of the predictions, the accuracy of the model was tested empirically with data on latrine density provided by the Ministry of Agriculture, Fisheries and Food. These test data were not used in the development of the model.

Tukey's honest significant difference (HSD) test was used as *a posteriori* comparison of means due to its reliability regarding data showing departure from the assumptions of normality and homoscedasticity of variances and the fact that it is a suitable test for small and/or unequal sample sizes (Zar 1984; SAS Institute Inc. 1985). Kruskal-Wallis one-way analyses of variance were followed by *a posteriori* Tukey-type test of means (Dunn 1964). Non-parametric Tukey-type multiple comparisons of means were also conducted following X^2 comparisons (Zar 1984).

Analyses of variance using blocks were conducted using the General Linear Modelling procedure in the SAS statistical package. All other analyses were conducted using Minitab. All means are expressed $\pm$ standard error and significance is defined at the 0.05 level.

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

Radio tracking has revolutionised the opportunity to gather ecological data, particularly on elusive nocturnal species such as the badger. However, the accuracy with which radio fixes resolve movement patterns is often coarse compared with the resolution required. Although direct observation has facilitated analysis of scent marking e.g. Macdonald (1979, 1980), Wells & Bekoff (1981) and Kruuk (1992), such observations are difficult and rely largely on chance encounters. Due to the notoriously poor spatial resolution of radio tracking (Macdonald & Amlaner 1980) and chance nature of direct observation, a technique for tracking badgers was required that would reveal the precise path taken by an animal. Spool-and-line tracking has been used successfully for this purpose on a wide range of mammals, e.g. Miles, de Souza & Póvoa (1981a), Berry *et al.* (1987) and Hawkins & Macdonald (1992). It involves attaching a spool of thread to an animal, releasing it and later following the thread. This technique in conjunction with a biomarker that would mark urine, faeces and anal gland secretions would enable a detailed examination of the pattern of scent marking by individual badgers, a prerequisite for understanding the process of bovine tuberculosis transmission from badgers to cattle. Understanding the role in territorial scent marking behaviour of individuals of known age, sex and social status would yield the type of information necessary to understand the function of territoriality and hence the function of group living in this species.

In this chapter the use of spool-and-line tracking and biomarkers in other studies is reviewed, together with a detailed account of the methodology behind the techniques used in the present study. Problems associated with spool-and-line tracking are discussed and data are presented that justify the use of the techniques in this chapter.

2.1.1 Spool-and-line tracking

Spool-and-line tracking, first described by Breder (1927), has been modified on several occasions since that date. Both Breder (1927) and Stickel (1950) developed 'trailing' techniques to study the activities of turtles (*Terrapene c. carolina*). Greegor (1980) was able to gage the extent of the home range of the armadillo (*Chaetophractus vellerosus*) by attaching a spool of polyester thread (455m long) to the tail of individuals. Miles (1976) developed a crude spool-and-line tracking device which was attached to mammals that had been captured in traps. This device enabled him to locate the nests and refuges belonging to these mammals, from which he could collect the silvatic triatomine bugs (Hemiptera: Reduviidae) that transmit *Trypanosoma cruzi*. Miles, de Souza & Póvoa (1981a) further improved the design of the tracking device and produced five different sized spool-and-lines (range 160-2300m long) enabling a greater diversity of mammals to be tracked as part of a study on the epidemiology of Chagas' disease (Miles, de Souza & Póvoa 1981b). Spool-and-lines were attached to a total of 263 edentates, marsupials, rodents and carnivores. For animals the size of the common opossum (*Didelphis marsupialis*) the spool-and-lines were attached laterally between the base of the thorax and the hips with strong adhesive tape. Animals smaller than *Didelphis* carried the spool-and-line mid-dorsally to prevent disturbance of their equilibrium. Of the 263 animals fitted with spools of line, 170 were retrieved, a success rate of 64.6%. The success rate obtained varied between the 16 species of

mammal tracked. Strictly terrestrial species inhabiting dry areas proved to be the easiest to track, although arboreal species were also successfully tracked. The major cause of the 93 failures was due to line breakage (over 50%), primarily because of simple design faults which could quite easily be improved.

Berry *et al.* (1987) improved on the spool-and-line design weaknesses of Miles (1976) to track both black and white eared giant rats (*Mallomys rothschildi*) and (*Hyomys goliath*) respectively. Spool-and-line tracking devices consisted of 1550m of fine terylene thread, enclosed in a plastic sheath with a hole at one end. These were attached laterally to study animals with a belt of sticky tape to prevent their removal and avoid impairing locomotion of the animal. The following day, the thread trail was followed and mapped by taking 16 point compass bearings at 5m intervals along the thread trail. Relocating the animals at daytime refuges enabled spool-and-line devices to be examined and replaced if the old one contained insufficient thread. These authors found it possible to estimate whether an animal was stationary (concentrated jumble), moving slowly (slack loops), or travelling fast (taut), from the pattern of thread deposition. The information obtained from the spool-and-line tracking demonstrated that these two superficially similar species are segregated ecologically, both spatially and on the basis of diet.

Anderson *et al.* (1988) used the same spool-and-line device as Berry *et al.* (1987) for a short term ecological study of the New Guinea spiny bandicoot (*Echymipera kalubu*). Design improvements included a largening of the exit hole for thread in the spool device which provided a freer passage of thread from the spool, thereby reducing fraying and breakage. Filing smooth the exit hole also reduced fraying. Finally, melting rather than glueing the spool and sheath together prevented the spool from shifting within the sheath, a problem experienced by Miles, de Souza & Póvoa (1981a). To determine distances travelled in different habitat types these authors also took compass bearings at 5m intervals along the thread trail. Although this technique provided a precise topological map of thread

trails, it did not provide information on the pattern of thread deposition between the 5m intervals and hence underestimated distance travelled in a similar manner to radio tracking. Another problem was that animals caught in traps by Berry *et al.* (1987) and Anderson *et al.* (1988) were retained during the day in a canvas bag and released at dusk. As the information gathered in both studies was derived from movements of the study animal during that night this raises the question of abnormality in behaviour of the study animals.

Hawkins & Macdonald (1992) have applied this technique to badgers. Spools consisted of 4km of polyester thread encased in a plastic carton that were attached to a collar on the animal so that they hung beneath the badger's neck. Fifteen badgers were equiped with spool-and-line devices and data collected from seven of these. Again the position of the thread was recorded at 5m intervals, providing information on habitat use.

Spool-and-line tracking has great potential to follow fine grained individual patterns of movement. Its application seems to suit studies on disease transmission being used for such purposes by Miles (1976) and Miles, de Souza & Póvoa (1981b). Another important application of the technique is for studying the pattern of scent marking behaviour of individual animals. For animals that transmit disease from their excretory products this technique is ideal for providing information on scent marking and disease transmission. Spool-and-line tracking has only a limited role in studies on habitat use, mainly because it provides no precise information on time budgeting in different habitats. Although Berry *et al.* (1987) suggested that the speed of movement could be deduced from the pattern of thread deposition this will probably be complicated by different vegetation types catching the thread at different frequencies, affecting the deposition of the thread. Combining spool-and-line tracking with radio tracking would overcome the problem above by providing a time frame for the spool-and-line data.

2.1.2 Biomarkers to locate urine and faeces

Fluorescent materials have been used as wildlife markers for many years e.g. Gast (1963), Frantz (1972), Evans & Griffith (1973) and Johns & Thompson (1979). Their uses include, bait identification, direct application to exterior of animal and the internal marking of the digestive tract and excretory products. Marking excretory products by feeding, injecting or implantation of various dyes has been used to study animal movement (New 1958, 1959; Kindel 1960; Brown 1961; Brown & Conaway 1961).

New (1958) placed dyed baits at bait stations on a grid which was examined for coloured scats. The location of coloured scats within the grid was used to determine distances moved by short-tailed shrew (*Blarina brevicauda*), deermouse (*Peromyscus leucopus*) and meadow mouse (*Microtus pennsylvanicus*). Davis, Emlen & Stokes (1948) and Seal & Bhattacharji (1961) also supplied food containing dyes that subsequently showed up in faeces or urine. The distributions of dyed faeces or urine indicated movements of animals and their home range size. The advantage of this technique for determining small mammal home ranges is that no trapping or handling is necessary, thus providing the minimal disturbance to both study animals and habitat. The disadvantage is that allowing animals to eat at bait stations provides no information about individual animals. It is also possible that the presence of baits may affect the movements of animals under investigation. Another major disadvantage of the technique is that it is inefficient in terms of time economy and accuracy when randomly searching for dye traces.

The problems associated with bait stations has largely been overcome by Brown & Conaway (1961), marking individual *Mus musculus* and *Microtus ochrogaster* by staining their urine with subcutaneously placed pellets of dye mixed with beeswax. However, although they stated that activity was not depressed by this operation, as measured by activity wheels, there is no information on the effects

of such an operation on the social activity of the rodents in the field. Randolph (1973) measured home range use by feeding marker bait to small mammals temporarily held in captivity, releasing them and later collecting marked faeces deposited in containers left around the study area.

Frantz (1972) developed a fluorescent baiting technique for estimating home range size while studying the behaviour of lesser bandicoot rats (*Bandicota bengalensis*) in India. This technique consisted of baiting with fluorescent pigment and examining the study area under ultra-violet illumination for fluorescing rat faeces, tracks and body rub-marks. Examining the study area under ultra-violet light saved time and increased the chances of detecting evidence of movement.

The biomarker techniques discussed so far have been applied mainly to determining home range size. Fluorescent pigments have been used by Lemen & Freeman (1985) to track in detail small mammals for up to 900m, providing data of a spatial quality similar to spool-and-line tracking. The fur of an animal would be saturated with pigment by placing the animal in a plastic bag containing pigment, gently shaking and releasing the animal during the night. The following night the area would be surveyed using an ultra-violet lamp and movements during the previous night recorded. The technique provides detailed information on home range, movement patterns, dietary requirements and habitat use, both vertical and horizontal.

2.1.3 Survival of *Mycobacterium bovis*

Locating excreta from infected badgers at night shortly after they were produced would enable contaminated grass samples to be removed for subsequent bacterial culture to establish the longevity and vertical distribution of *M. bovis* on pasture. The Ministry of Agriculture, Fisheries and Food (1979) report that when urine from naturally infected badgers was tipped on to pasture during the winter,

large numbers of organisms were recovered after one week and scant numbers after four weeks. However, when this trial was repeated in the summer, no organisms were recovered after only three days of exposure. A similar pattern was reported for faeces, with faeces remaining infected after one month in the winter and becoming negative after two weeks of exposure in the summer.

Clearly the conditions experienced during the summer period are deleterious to the organism, resulting in poor survival of *M. bovis*. However it is not understood which particular climatic conditions or combination of conditions is responsible for low and/or high survival, nor how the position of excreta on pasture and grass length are related to their survival. Locating excreta on pasture, in particular infected urine, would enable the vertical distribution of *M. bovis* on pasture to be investigated and how this might be affected by climate. The timing of excretion by badgers during the night may be important in relation to the number of viable organisms present on pasture the following morning when cattle begin grazing. Thus establishing how quickly organisms are killed in the first few hours after being void is essential in understanding the potential risk from excreta on pasture. Although the survival of *M. bovis* was not investigated in the present study, the ability to accurately locate all excreta from individuals would provide a useful research technique for subsequent studies on the survival of *M. bovis*.

2.2 Methods

2.2.1 Construction of the spool-and-line

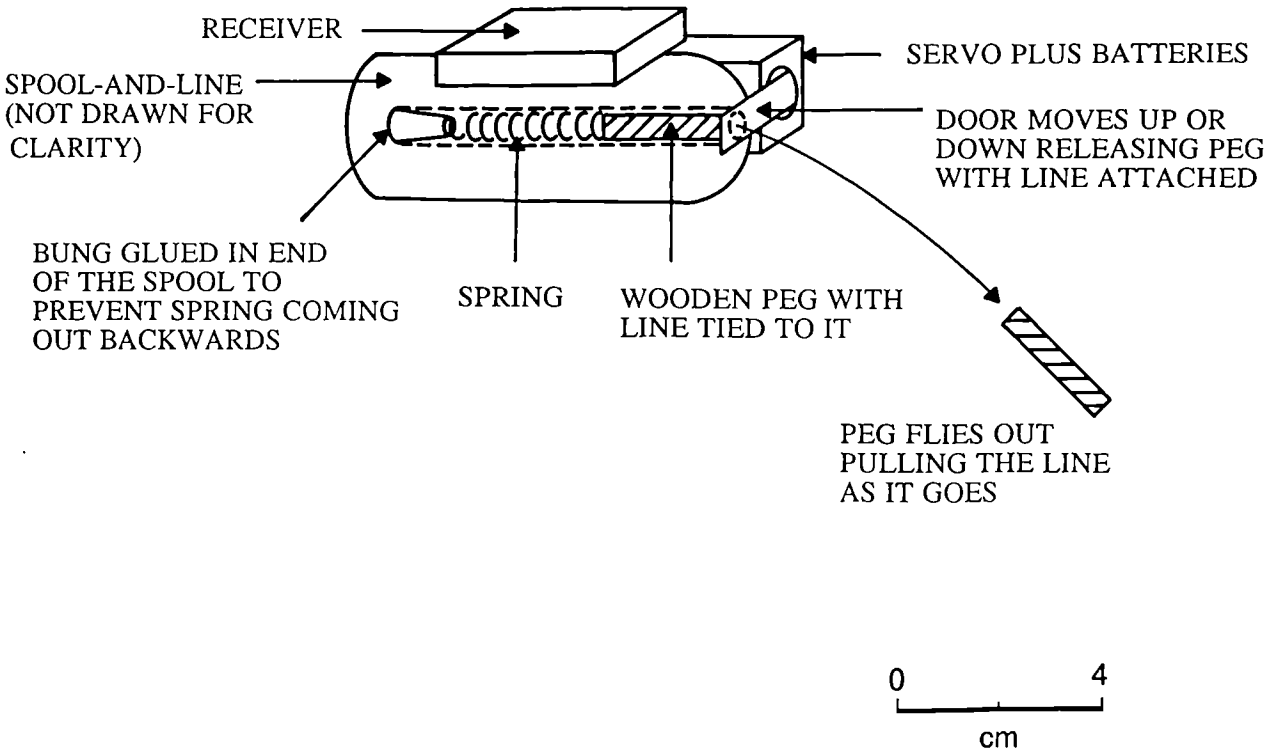
The original plan for the construction of the spool-and-line was to develop a device which would allow the line to be released two or three nights after capturing the badger. This would then overcome any potential changes in behaviour following capture and handling. Two devices were tested that would control the

release of the line. One involved a radio receiver mounted on the spool-and-line connected to a servo motor (Figure 2.1). A metal plate attached to the servo motor covered the exit hole for the line and held a wooden peg under tension. On receipt of a signal the servo motor rotated causing the metal plate to uncover the exit hole releasing the wooden peg and the line attached to it. Thus as the badger left the sett two or three nights post-capture this system would be operated. However, this proved to be too bulky and battery life was minimal with the equipment involved.

The second design was based on an electronic delayed timing device. The electrical side was relatively simple and the technique overcame all of the above difficulties, although due to reducing the size of the spool-and-line by removing the additional features controlling line release, no reliable mechanical firing mechanism could be developed. As a result of these problems, it was decided not to include any device to delay release of the line.

The spool-and-line finally adopted was similar to that used by Anderson *et al.* (1988), and did not include any triggering or timing device. Therefore the badger had to be tracked the night following capture. Since this badger population has been trapped extensively as part of a long term study, it was felt that any stress following capture was likely to be minimal, and so following the animal on the night post-capture was unlikely to give aberrant data. However, this assumption was tested as described below. For the initial trials 1,500m lines were used, but these were too short to provide a full night's data. These were replaced by 3,000m lines; these consisted of bleached white tri-lobal polyester thread (breaking strain 0.9kg) which was enclosed in a polypropylene centrifuge tube (39mm internal diameter) with a 10mm hole at one end. Centrifuge tubes were cut to 80mm from an original length of 130mm, allowing a gap within the tube of approximately 10mm between the top of the spool and the exit hole. Spools of line are supplied by James Pearsall & Co. Ltd., Tancred Street, Taunton, Somerset TA1 1RY. The

Figure 2.1 The firing mechanism of the original spool-and-line which it was hoped could release the line two or three nights post-capture, although due to difficulties a device delaying the release of the line was not included.



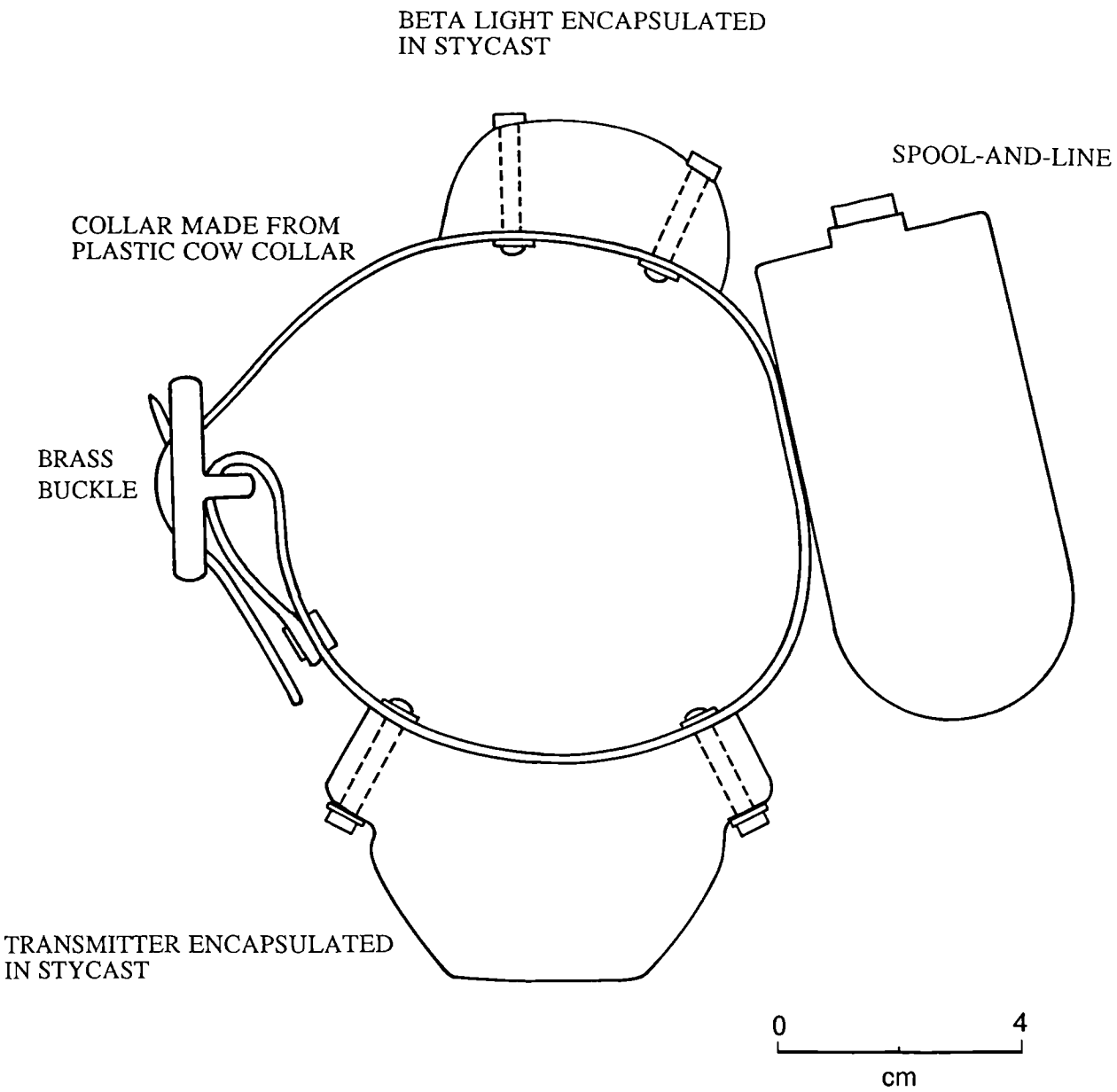
hole created in the base of the centrifuge tube was filed smooth to reduce friction between the line and the sides of the hole, allowing the line to run freely from the device as the badger moved. The base of the spool was attached to the mouth of the centrifuge tube with a soldering iron and again filed smooth to prevent the line fraying. This method was prefered to using glue as it excluded the possibility of glue touching the line and causing subsequent breakage.

The spool-and-line in its centrifuge tube weighed 70g and was fitted parallel to a collar which also carried a radio transmitter (Figure 2.2). The centrifuge tube containing the spool-and-line was attached using insulating tape; this would allow the device to fall off within a few days if the animal was not later recaptured. The complete package, including collar, spool-and-line, beta-light and transmitter, weighed 211g and so was well within the guidline of 5% of the body weight suggested by Macdonald (1978). Radio tracking was undertaken simultaneously with the spool-and-line tracking, with a badgers's position recorded at 15min intervals, to provide a time frame for the spool-and-line data.

2.2.2 Capture and handling procedures

Selected badgers were caught in cage traps (Cheeseman & Mallinson 1980) which were not set until the middle of the night. Traps were examined at first light and selected animals sedated by an intra-muscular injection of ketamine hydrochloride (Vetalar^{R}, Parke Davis), while still in the trap, before being fitted with a spool-and-line. Anaesthetised badgers were transferred to a smaller holding cage to recover. Movement within the holding cage was restricted compared to the cage traps, reducing the amount of tangling of the line around the badger as the animal recovered from anaesthesia. The smaller mesh size of the holding cage also prevented the spool-and-line device from contacting the ground, preventing soil from entering the device through the exit hole.

Figure 2.2 The arrangement of the spool-and-line on the radio-collar; the spool-and-line is attached with insulating tape with the opening facing towards the transmitter, and will soon fall off the collar.



The badger was released at the hole closest to the capture point and the free end of the thread attached to vegetation in the vicinity of the hole. Care was taken when releasing the badger to ensure that the line was running freely. Although tangling of the badger was reduced in the holding cage, ocassionally it proved necessary to insert a stick into the cage to remove line around limbs. Before releasing the badger the line hanging directly beneath the spool-and-line device was brought to the side of the cage and held, ensuring that as the badger ran towards the hole the line was running freely and did not become entangled around its limbs, since this would probably break the line.

Although trapping in the middle of the night reduced capture rate, badgers caught this way were returning from their feeding grounds with a full stomach. This was important since urination and defecation were likely to proceed as normal the following night. Badgers caught emerging from the sett would not have fed for approximately 36 hours prior to being tracked. This reduced food intake could result in a deviation from normal behaviour and a lack of or reduced rate of urination/defecation during the early part of the night that they are tracked. Whilst ensuring that the excretory behaviour was not affected on the night of tracking, this protocol also meant that the period in captivity was as brief as possible and handling procedures kept to a minimum, thereby ensuring that there was the minimum impact on the animals behaviour the following night. All badgers that were spool-and-line tracked had previously been fitted with a radio collar, and so the spool-and-line was attached to an existing collar, thereby causing the minimum of additional stress.

To check whether the capture and handling procedures affected the animal's behaviour the following night, an activity recorder was placed near to the main sett of one study group for the summer of 1991 to record both emergence times and return times to the sett. The activity recorder was set so as to record only badgers above ground in the vicinity of the main sett. Therefore it was also necessary to record daytime badger positions to ensure that the first signal recorded was from an

animal leaving the main sett, and the last recorded signal for a particular animal was due to the animal entering the main sett and not just leaving the area. The recorder was on continuous sweep to prevent animals emerging and returning undetected. Data were collected nightly from all collared members of the social group (n=9) during this season. This included badgers that carried just radio collars plus the animals that were being spool-and-line tracked. Within this season the emergence times and time away from the main sett were compared for individuals spool-and-line tracked to randomly selected nights for the same individuals when they had not recently been handled.

2.2.3 Administration of biomarker

A biomarker was needed that would show up in both urine and faeces and could be detected at night, so that the pattern of urination and defecation by badgers could be studied. As described earlier, Frantz (1972) used baits marked with fluorescent pigments and examined the study area at night under ultra-violet illumination for fluorescing rat faeces, tracks and body rub-marks. This technique was considerably more efficient in locating dye traces than many other techniques e.g. Davis, Emlen & Stokes (1948), New (1958) and Seal & Bhattacharji (1961). Therefore, various fluorescent dyes were investigated using a captive colony of badgers, but none proved as easy to locate as fluorescein. Fluorescein has a number of other practical advantages: it is freely soluble in water and produces an intense yellow/green fluorescence perceptible down to 0.04ppm. Webb, Fonda & Brouwer (1962) found that, following oral administration, fluorescein and its mono- and dihalogenated derivatives could be detected in the urine and bile of rats and that the basic fluorescein structure was not degraded in the animals, as shown by the high (90-100%) recovery of fluorescein from excreta. They also found that they were absorbed at low dose levels (0.5 to 5.0mg kg^{-1} body weight) as indicated by their

detection in either the bile, urine or both within 2-4 hours after ingestion. The tri- and tetrahalogenated and tetrachloro-tetrabromo derivatives generally were not detected in the urine at low dose levels and in fact the latter was not found in the urine at any dose level. The higher halogenated derivatives were excreted in significantly shorter times than that required for fluorescein or its dihalogenated derivatives (Webb, Fonda & Brouwer 1962). Iga, Awazu & Nogami (1971) reported that the presence of halogen groups had great influence on the biliary excretion of the fluorescein dye. The larger the number of halogen groups the larger the excretion ratio, i.e. a greater proportion is excreted in the bile and ultimately in the faeces than in the urine. This is due to the halogen groups increasing both molecular weight and polarity. These findings and the fact that fluorescein has been demonstrated to be non-toxic (Smart 1984) led me to investigate the use of this dye as a biomarker to help locate badger urine, faeces and anal gland secretions at night using a hand-held portable ultra-violet lamp. The lamp chosen was one that emits ultra-violet light of 366nm wavelength; this will not kill bacilli since ultra-violet light is not bactericidal above 330nm. Fluorescein dye was supplied by Holiday Dyes & Chemicals Ltd., P.O. Box B22, Leeds Road, Huddersfield HD2 1UH.

For the original trials on captive badgers, held in a pen of $1600m^2$ surface area, fluorescein LT was presented orally in a bait. However, this method was non-selective and also produced unnecessary contamination with dye in the area of bait release, so a different technique was needed for field use. Two captive badgers were used to compare subcutaneous injection and oral administration of 10ml of a $0.38g\ ml^{-1}$ solution of fluorescein LT prepared in sterile water. For the oral administration a dog catheter (length 50cm, outer diameter 2.8mm) was used to inject the dye directly into the stomach of a 12-year-old sow; a 2-year-old sow was used for the subcutaneous injection with the dye injected into the flank of this animal. The two badgers were then held in seperate cages (1.2m x 0.9m x 1.1m)

and supplied with bedding, water and dead day-old chicks *ad libitum*. Each cage was raised off the ground and a polythene sheet placed underneath. Pockets were formed in the sheet using broom handles so that successive urinations were less likely to mix. The sheet was examined at three hourly intervals for the following five days and any urine and faeces collected. The quantity of urine produced at each urination varied considerably, ranging from 5.5 to 85ml. The presence of fluorescein in the urine, faeces and anal gland secretion was revealed at night using the ultra-violet lamp.

2.3 Results

2.3.1 Badger emergence times

The emergence times and time away from the sett are shown in Table 2.1. Both emergence times and time away from the sett the night following capture and handling were compared with randomly selected data from the same animals in the same season when they had not recently been handled. A paired sample t-test revealed no significant differences in either emergence times ($t=0.58$, d.f.$=16$, $p>0.5$) or the time away from the sett ($t=-1.49$, d.f.$=16$, $p>0.1$).

2.3.2 Retention time of fluorescent dye

Following subcutaneous injection, fluorescein was detectable in the urine for over 93hrs after injection and more than 144hrs for the faeces; the badger was still excreting fluorescein in its faeces after it had been returned to the captive colony. In comparison, with the catheterised badger the first negative urine occurred after 63hrs and no more fluorescein was detected in the urine after 84hrs. Faeces of this animal no longer contained the dye after 45hrs. Clearly subcutaneous injection

Table 2.1 Comparison of emergence times (time in minutes from sunset) and duration away from the sett (min), between nights post-capture during the summer season, with randomly selected nights for the same animals in the same season when they had not been recently handled.

	Spool-and-line	Control	n
Mean emergence time	64.2 ± 15.7	53.6 ± 10.9	9
Mean duration from sett	351.4 ± 17.9	390.0 ± 21.0	9

Sunset times prepared by H.M. Nautical Almanac Office, Royal Greenwich Observatory (longitude W000° 00, latitude N52° 00).

resulted in the longest retention of the fluorescein, and was subsequently used for the field trials.

2.3.3 Effect of biomarker on *Mycobacterium bovis*

Trials were undertaken to examine the effect of a 0.38g ml^{-1} solution of fluorescein LT on the growth of *M. bovis*. A dilution series (10^{-1} to 10^{-4}) of *M. bovis* was prepared in saline to give low numbers of organisms present in the dilutions. From each dilution, 0.1ml was added to 0.9ml of each of a) saline, b) urine and dye, c) urine alone. The second sample (urine and dye) was obtained by collecting the first urination from a badger subcutaneously injected with 10ml of a 0.38g ml^{-1} solution of fluorescein LT. From each treatment, 0.25ml was sown on to each of three 7 H 11 slopes. This initial trial (Table 2.2) showed that a 0.38g ml^{-1} solution of fluorescein LT appeared to inhibit the growth of *M. bovis*.

To establish the degree of inhibition of *M. bovis* by a 0.38g ml^{-1} solution of fluorescein LT, the experiment was repeated using a higher dose of *M. bovis* with dilutions ranging from 10^{-1} to 10^{-6}. Colonies were present on the slopes of the saline treatment down to a dilution of 10^{-5} and 10^{-4} for the urine treatment, although, as with the previous experiment, *M. bovis* could not be isolated in the presence of the dye (Table 2.3). It was possible that the concentration of fluorescein present in the urine was critical and so a range of different dye concentrations were examined. As with the above example 10ml of each dye concentration was subcutaneously injected into a different badger and urine containing the dye collected. Table 2.4 shows that considerable numbers of bacilli were recovered when a 0.22g ml^{-1} solution of fluorescein LT was used, and that more bacilli were recovered using fluorescein LT compared to fluorescein LTS. The former is a technical grade dye that contains fewer impurities; these results suggest that a 0.22g ml^{-1} solution of fluorescein LT is suitable for field use.

Table 2.2 Effect of fluorescein dye on the isolation of *M. bovis* from badger urine (low dose of *M. bovis* used).

Sample	Dilution	Replicate		
		1	2	3
Saline	10^{-1}	12b/c	4b/c	14b/c
	10^{-2}	-	3b/c	-
	10^{-3}	-	-	-
	10^{-4}	-	-	-
Urine +dye	10^{-1}	-	-	-
	10^{-2}	-	-	-
	10^{-3}	-	-	-
	10^{-4}	-	-	-
Urine only	10^{-1}	5b/c +1col	3b/c	3b/c
	10^{-2}	-	-	1b/c
	10^{-3}	-	-	-
	10^{-4}	-	-	-

Key: b/c= "breadcrumb"(colony in condensation water); col= colony on slope; - = no colonies present.

Table 2.3 Effect of fluorescein dye on the isolation of *M. bovis* from badger urine (high dose of *M. bovis* used).

Sample	Dilution	Replicate		
		1	2	3
Saline	10^{-1}	+++	+++	+++
	10^{-2}	+++	+++	+++
	10^{-3}	++	++	++
	10^{-4}	b/c	b/c	-
	10^{-5}	+	-	-
	10^{-6}	-	-	-
Urine+dye	10^{-1}	-	-	-
	10^{-2}	-	-	-
Urine only	10^{-1}	+++	+++	+++
	10^{-2}	+++	++	++
	10^{-3}	+	b/c	b/c
	10^{-4}	+	+	+
	10^{-5}	b/c	-	-
	10^{-6}	-	-	-

Key: +++ = >50 colonies on slope; ++ = 10-50 colonies; + = < 10 colonies; b/c = "breadcrumb" (colonies in condensation water); - = no colonies present.

Table 2.4. Growth of *M.bovis* in badger urine and fluorescein three weeks after inoculation.

Dilution	Saline	Urine only			Urine+dye				
					Fluorescein(LT)		Fluorescein(LTS)		
					0.12g ml^{-1}	0.22g ml^{-1}	0.12g ml^{-1}	0.22g ml^{-1}	0.38g ml^{-1}
10^{-1}	+++	+++	+++	+++	+++	+++	-	+++	+++
	+++	++	+	+++	-	+++	-	+++	-
	+++	+	-	+++	-	+++	-	+++	-
10^{-2}	+++	+	+++	+++	+++	+++	+++	+++	-
	+++	+	+++	+++	+	+	+++	-	-
	+++	-	+	+++	-	+	-	-	-
10^{-3}	+	+	++	++	++	++	++	+	+
	+	-	+	++	++	++	-	-	-
	-	-	+	++	-	+	-	-	-
10^{-4}	++	+	-	+	+	+	-	+	-
	+	+	-	+	-	-	-	-	-
	-	-	-	+	-	-	-	-	-
10^{-5}	-	+	-	+	+	-	-	+	-
	-	-	-	+	+	-	-	-	-
	-	-	-	+	+	-	-	-	-
10^{-6}	+	+	+	+	-	-	-	+	-
	-	+	-	+	-	-	-	-	-
	-	-	-	+	-	-	-	-	-

Key: +++=>100 colonies on slope; ++=20-100 colonies; +=1-20 colonies; -=no colonies isolated.

2.3.4 Relationship between period of above ground activity and trail length

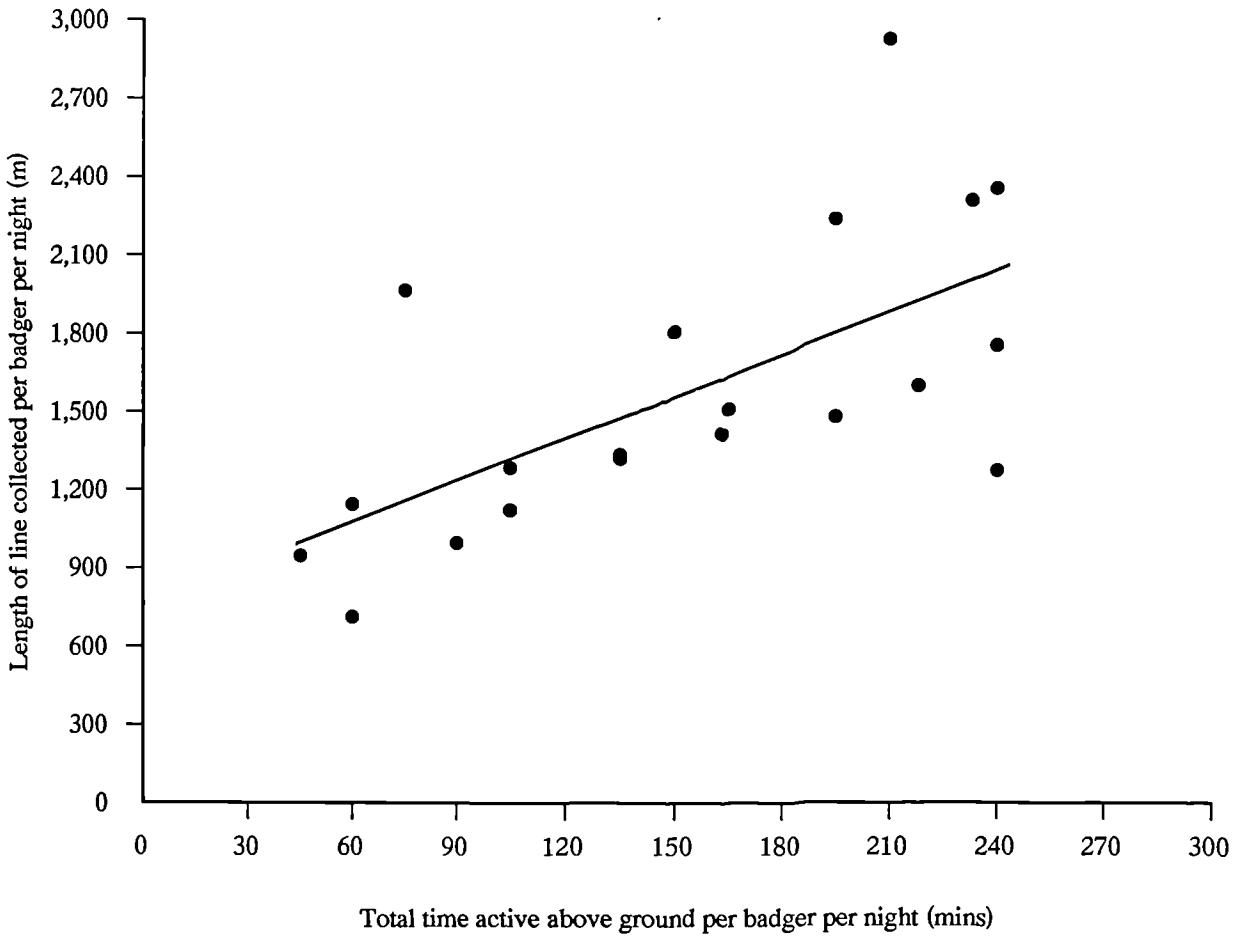
In Figure 2.3, the period of above ground activity was compared with the length of line collected per night for badgers throughout the year. This revealed a significant relationship between these two variables ($F=12.71$, $R^2=38.1$, d.f.$=1,18$, $p=0.002$).

2.4 Discussion

Spool-and-line tracking can show the precise route taken by an individual animal and when used in conjunction with a biomarker it allows the location of the urination and defecation sites of specific animals. There are, however, a number of problems associated with this technique.

According to Berry *et al.* (1987), the pattern of thread deposition provides an indication of the time spent in each area. Figure 2.3 indicated this to be true in the present study, although the pattern of thread deposition is likely to vary according to the particular habitat type, as different types of vegetation catch the thread at different frequencies, thereby making it very difficult to quantify. Therefore, in the present study radio tracking was used in conjunction with the spool-and-line technique to provide a time element for the analysis of the animals' movements. Other problems relating to the spool-and-line technique are more difficult to resolve. Grazing animals can cause problems by dragging the thread from its original position with their feet and also by eating it. Animals which forage on pasture, such as foxes and hedgehogs (*Erinaceus europaeus*), as well as other badgers, can also alter the position of the thread. Wind can distort the position of the thread as it is being released and also when on the ground, especially in open fields where there are relatively few points of attachment. These problems are minimised by following the thread during the night and at a distance behind the

Figure 2.3 Relationship between the total nightly time spent active by badgers above ground and the total length of line collected per badger per night.



badger that makes it unlikely for wind and other animals to distort the line before excreta are located. The major problem of distortion comes from cattle, especially during their major feeding bout in the morning. For this reason the position of the line was recorded on to a map and collected at first light, beginning with those fields known to contain cattle.

Recording the trail of line directly on to a map was preferred to taking bearings used in other studies, e.g. Berry *et al.* 1987, Anderson *et al.* 1988, Hawkins & Macdonald 1992, since these authors experienced problems in taking accurate bearings and also because of the time involved in taking bearings. Due to the possible displacement of line on the ground by cattle, a simpler, more time efficient technique was required to record the position of the line. Given the abundance of visual reference points on the study site, this way of recording was relatively easy.

Another difficulty relating to this type of tracking is the short term scope of each device. A badger could only carry a certain amount of thread, which was generally used up within one night. Thus the major potential problem associated with this technique was that of following the animal on the night post-capture and the possible behavioural abnormalities this may induce. However, since this population is under intensive study and regularly trapped, any affect on an animal's behaviour post-capture was likely to be minimal. This was supported by the lack of a significant difference between emergence times and time away from the sett between nights post-capture and nights when the same animals had not recently been handled. Thus coupled with only capturing animals on their return to the sett, ensured that this technique gave an accurate measure of an animal's movement and excretory behaviour.

Fluorescein has been extensively tested on a variety of micro-organisms for mutation and DNA alteration. The DNA-cell binding test suggested that this dye was a possible mutagen on metabolic activation (Kubinski, Gutzke & Kubinski

1981). Nestmann *et al.* (1979) and Douglas *et al.* (1980) reported rhodamine B to be mutagenic in *Salmonella* and mammalian cells and demonstrated that the mutagen was an impurity in the technical grade dye employed. This supports the observation that more bacilli were recovered using the technical grade dye fluorescein LT which contains fewer impurities than fluorescein LTS. This result has important implications, because the concentration of impurities in commercial dyes may vary with both manufacturer and dye batch, and so each batch of dyes should be tested before it is used to locate urine patches from which bacilli are to be cultured. Unfortunately during the period of data collection very few infected animals were present on the study site and so survival of bacilli on pasture was not investigated. However, this technique provides a valuable technique for future research on this topic.

Apart from the bacteriacidal properties of the 0.38g ml^{-1} solution of fluorescein, the 0.22g ml^{-1} solution was more suited to the spool-and-line technique used, as dye would be excreted over a shorter time period, reducing the possibility of confusion over the origin of excreta when following subsequent animals. This possibility was minimised by leaving several days between the tracking of successive animals from the same group.

By altering the number of halogen groups on the fluorescein structure it is possible to alter the length of time the dye is excreted from an animal (Webb, Fonda & Brouwer 1962). By the same procedure it is also possible to control the proportion of dye excreted in the urine and faeces and even totally block excretion in the urine using certain fluorescein derivatives (Iga, Awazu & Nogami 1971). This level of control over the excretion characteristics of the dye suggests that fluorescein is particularly suitable for studies requiring biomarkers.

Thus the combination of spool-and-line tracking, used in conjunction with radio tracking, and subcutaneous injections of fluorescein provide a valuable means of studying the precise nightly movement behaviour of individual badgers, and

monitoring their nightly urination and defecation behaviour. In chapters 3 and 4 I will show how this technique has furthered our understanding of these aspects of badger behaviour.

CHAPTER 3

SCENT MARKING AND SPATIAL BEHAVIOUR

3.1 Introduction

Members of the Carnivora do not often live in groups, with only about 10-15% of all species aggregating at some time outside the breeding season (Bekoff, Daniels & Gittleman 1984; Gittleman 1984). Several studies of the larger Carnivora have shown that there is a clear relationship between the social organization of the species and the way it exploits its food supplies (Kruuk 1972, 1975). This relationship is based on the extent to which an animal has to collaborate with conspecifics in order to catch prey.

Badgers are unusual amongst social carnivores in that they show no individual co-operation during foraging or group territorial defence, and the factors leading to group living in this species are unclear. The advantages of group living and the possible reasons for its evolution, such as the increase in foraging efficiency, defence of captured prey and defence against predators, cannot be applied to badgers (Kruuk 1989). In fact Cheeseman *et al.* (1993) found that following the removal of badgers, mortality of the re-colonising badgers was lower than for badgers in other social groups. These authors also found a significant increase in cub growth rates and adult body weights following badger removal, although these differences diminished as group sizes reached previous levels. Living in large social groups has also been shown to yield no net reproductive gain to adult male or female badgers, with productivity declining per adult with increasing group size (Cresswell *et al.* 1992).

The pattern of scent marking and territorial defence by social carnivores has generally been studied at the group rather than the individual level e.g. Kruuk (1978), Macdonald (1980) and Gorman (1990). Recently, some information on individual behaviour patterns has been obtained for coastal otters (*Lutra lutra*) (Kruuk 1992). The role of individuals in group territorial defence is generally poorly understood, however, analysing the role of individuals within the social group is particularly important in helping to understand the evolution of group living and territoriality in mammals.

There are two main theories which attempt to explain the evolution of group living and territorial behaviour in badgers. One states that the major function is the defence of food resources (Kruuk 1978; Kruuk & Parish 1982), whilst the other states that the evolution of group living has evolved to allow males to monopolise access to oestrous females (Roper, Shepherdson & Davies 1986). Whilst these theories are not mutually exclusive (Cresswell *et al.* 1992), they should lead to very different individual patterns of behaviour. For the food hypothesis, the following should hold true:-

(a) Since food is limiting (Cheeseman *et al.* 1987, 1993), territorial defence of food resources should be undertaken by all adult and yearling members of the social group, especially during summer when food availability is lowest, and the autumn, when badgers lay down winter food reserves.

(b) Territorial defence of food supplies should be most pronounced in females (Trivers 1972; Wrangham 1982).

(c) To avoid aggression from within the social group during periods of food shortage, and in particular to minimise the risk of infanticide (Cresswell *et al.* 1992) or the deleterious consequences to their survival and/or future breeding success following expulsion from their natal group (Roper, Shepherdson & Davies 1986), cubs should not advertise their presence to other group members.

For the sex hypothesis, the following should hold true:-

(a) Adult male badgers should increase boundary patrolling and territorial scent marking to restrict access to oestrous females by neighbouring males, particularly in the late winter and spring, since matings at this time of year account for 65% of total autumn blastocysts in yearling sows and 71% in older sows (Cresswell *et al.* 1992).

(b) Dominant boars should exhibit preferential access to oestrous sows, probably by some form of mate guarding.

(c) Younger boars should attempt to maximise their reproductive success by making most extra-territorial forays to search for oestrous sows in other groups, especially during the winter/spring.

To evaluate these two hypotheses, spool-and-line tracking was used to follow the nightly movements of individual badgers of known age, sex and social status, and fluorescein dye used to monitor their pattern of fecal, urine and anal secretion deposition. The pattern of bite wounding in badgers was used to examine the level of aggressive behaviour directed towards cubs, to evaluate the food hypothesis which suggested that cubs should maintain a low profile during periods of high food competition.

3.2 Methods

3.2.1 Measurement of movement patterns

The line laid each night was collected, cleaned, dried and weighed, and the distance travelled determined from a known weight:length ratio. Distances travelled within specific areas of the territory were calculated using a bit pad (Summagraphics^{R} Bit Pad^{R} Plus) and 1:2500 maps to measure the proportion of the trail within 100m of the main sett and within 50m of the territory boundary. For one of the five social groups, these two zones overlapped at one place; movements

within the area of overlap were excluded from subsequent analyses. In addition, distance specifically travelled along the boundary path was recorded. Area of nightly minimum convex polygon range overlap into neighbouring territories was also determined using a bit pad.

For each night, the number of latrines visited and the number of urinations, defecations and anal secretions were recorded. Taking account of the number of individuals within each age and sex category for each of the five groups and using the mean number of urinations, defecations, latrine visits and anal gland secretions for each age and sex category, the mean number of each of the above were calculated per group per night. Due to a low capture, yearling females were excluded from these calculations for each season. All movements were recorded on a map of scale 1:2500 for subsequent analysis of the pattern of range use (Figure 3.1). From this, a nightly minimum convex polygon range size was calculated.

3.2.2 Quantification of bite wounding

To look at the pattern of bite wounding in badgers, data were used from carcasses collected between March 1988 and March 1990 from Avon, Devon, Dorset, Gloucestershire and Wiltshire. Bite wounds were scored as absent, minor, and moderate/extensive on each of four regions (head, neck, rump, elsewhere) (Cresswell *et al.* 1992).

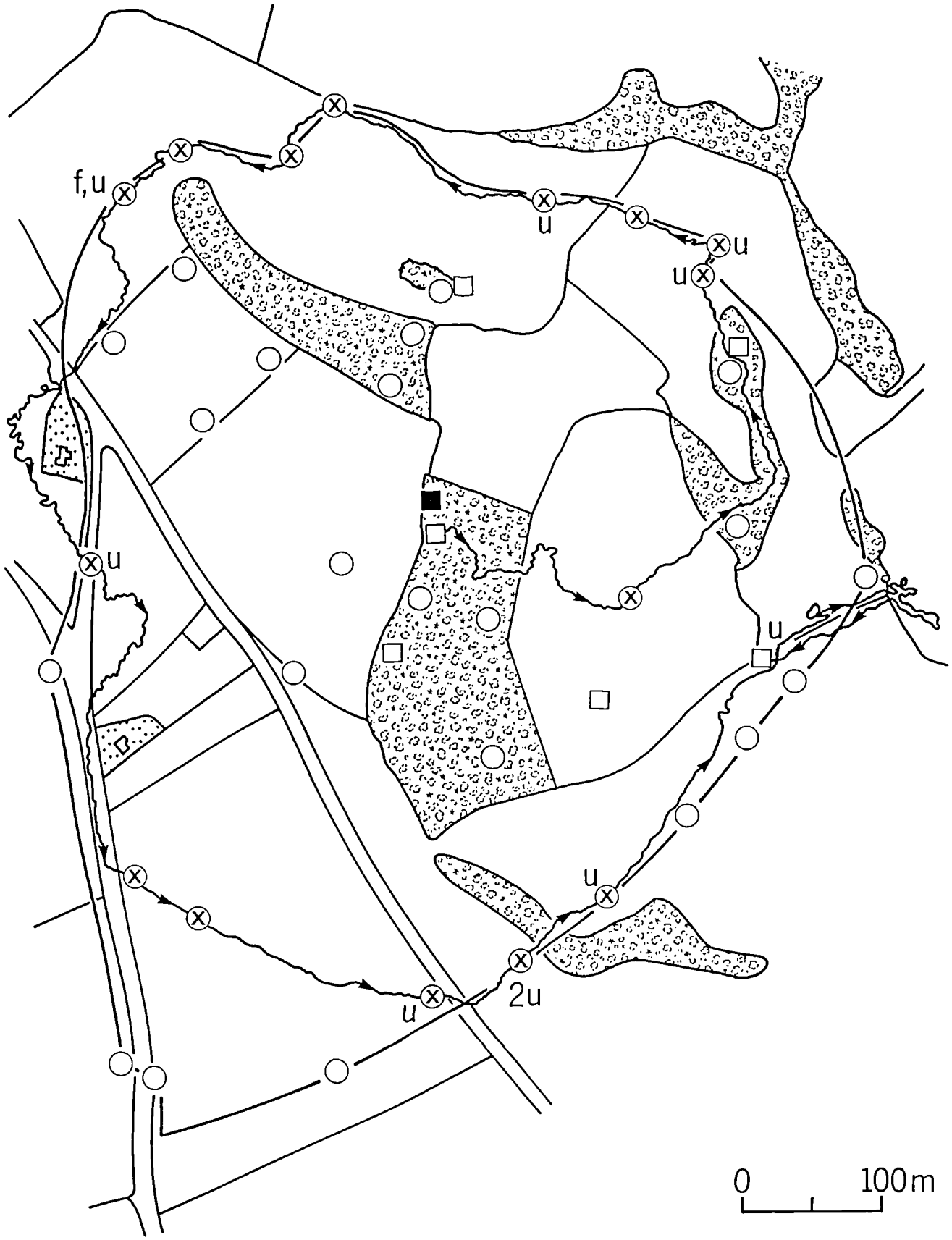
3.3 Results

3.3.1 Study animals

After a trial period to develop the technique, the data collected between June 1991 and May 1992 were used in this analysis. The number of complete spool-and-

Figure 3.1 Spool-and-line trace for (a) an adult male in September, and (b) an adult female in September from the same social group; arrows denote the direction of travel. Woods and roads are marked, as are gardens (stippled); all other areas are pasture. A solid square marks the main sett, open squares other setts, the thicker solid line the territorial boundary, and circles latrines. Latrines that were visited are marked with a cross, as are setts at which the animal went below ground and re-emerged; a - anal gland secretion left by the focal animal, f - faeces, u - urine.

(a)



(b)

line nights, and number of different animals tracked (n) for each season, were: spring 24 (n=21), summer 22 (n=17), autumn 21 (n=17), winter 26 (n=21). During the year the number of different animals tracked within each age and sex category was: adult males 9, adult females 15, yearling males 7, yearling females 6, male cubs 6, female cubs 7. Owing to the low capture of yearling females in the summer, autumn and winter, these animals were omitted from the analyses during these seasons.

3.3.2 Seasonal pattern of urination

One-way analysis of variance revealed that adult males urinated significantly more times per night than the other categories during the summer ($F_{(category)}=12.19$, d.f.=4,12, $p<0.001$, Tukey's HSD). Although frequency of urination by the groups reached a peak in the summer (Table 3.1), no significant seasonal variation was detected (one-way ANOVA, $F_{(season)}=2.75$, d.f.=3,16, $p>0.05$). One-way analysis of variance of ranks revealed that during the autumn adult males urinated significantly more on the territory boundary than the other categories ($F_{(category)}=9.09$, d.f.=4,12, $p<0.01$, Tukey's HSD), and adult males urinated significantly more times per night along the territory boundary in winter (one-way ANOVA of ranks, $F_{(category)}=6.33$, d.f. 4,15, $p<0.01$, Tukey's HSD). Female badgers of all ages urinated significantly more than adult males at hinterland latrines in the spring (one-way ANOVA of ranks, $F_{(category)}=4.14$, d.f.=3,16, $p<0.05$, Tukey's HSD). The number of boundary urinations deposited per group per night in the summer and autumn were significantly greater than in the winter season (one-way ANOVA, $F_{(season)}=6.31$, d.f.=3,16, $p<0.01$, Tukey's HSD). This was not the case for hinterland urinations, where there was no significant seasonal variation (one-way ANOVA, $F_{(season)}=1.99$, d.f.=3,16, $p>0.05$).

Table 3.1 Seasonal pattern of boundary urinations i.e. at boundary latrines and/or on a boundary run (above) and hinterland urinations (below) by different age and sex classes; figures for individual age and sex classes are the mean number per badger per night ± s.e and for the groups are the mean number per group per night ± s.e.

	Spring	Summer	Autumn	Winter
Adult males	0.44 ± 0.32	3.00 ± 4.24	4.00 ± 5.66	1.00 ± 0.58
	0.67 ± 0.23	4.00 ± 2.83	1.50 ± 0.71	1.67 ± 2.04
Adult females	0.57 ± 0.62	0.63 ± 0.43	0.25 ± 0.22	0
	1.86 ± 0.37	2.25 ± 1.52	0.60 ± 0.45	1.00 ± 0.68
Yearling males	0.67 ± 0.41	1.00 ± 1.41	1.00 ± 1.41	0
	1.17 ± 0.74	0.67 ± 0.41	0.50 ± 0.71	1.00 ± 0.67
Yearling females	0.25 ± 0.29	—	—	—
	2.00 ± 0.47	—	—	—
Male cubs	—	0	0	0
	—	0	0.25 ± 0.29	0.33 ± 0.41
Female cubs	—	0	0	0
	—	0	0.50 ± 0.33	0.25 ± 0.29
Groups	4.34 ± 0.79	8.22 ± 1.89	7.60 ± 1.96	1.20 ± 0.55
	11.60 ± 2.25	16.67 ± 4.41	8.43 ± 1.56	10.49 ± 1.99

3.3.3 Seasonal pattern of defecation

Analysis of variance revealed no significant difference in the mean number of defecations per night between age and sex categories in any season; similarly, there were no significant seasonal variations within the categories nor in the number of defecations per group per night (one-way ANOVA, $F_{(season)}=1.72$, d.f. = 3,16, $p>0.05$). No significant differences were detected in the number of boundary or hinterland defecations per night between the categories in any season, although yearling males deposited a greater proportion of their faeces on the boundary in the spring than adult females (one-way ANOVA of ranks, $F_{(category)}=4.88$, d.f.=3,16, $p<0.05$, Tukey's HSD). No seasonal differences in boundary or hinterland defecations were detected within the age and sex categories (Table 3.2), although in the summer the number of boundary defecations per group per night was significantly greater than in the autumn and winter (one-way ANOVA, $F_{(season)}=6.10$, d.f.=3,16, $p<0.01$, Tukey's HSD), while the nightly number of hinterland defecations per group in the autumn and winter was significantly greater than in the spring and summer seasons (one-way ANOVA, $F_{(season)}=9.30$, d.f.=3,16, $p<0.001$, Tukey's HSD).

3.3.4 Seasonal pattern of latrine visits

Summer was the only season in which there was significant variation in the overall pattern of visits to latrines (i.e. boundary and hinterland combined), with adult males visiting significantly more latrines per night than all other categories except yearling males (one-way ANOVA, $F_{(category)}=3.31$, d.f.=4,12, $p<0.05$, Tukey's HSD). Adult males also visited significantly more boundary latrines per night in the autumn than the other categories, again except yearling males (one-way ANOVA, $F_{(category)}=5.09$, d.f.=4,12, $p<0.05$, Tukey's HSD), and adult males

Table 3.2 Seasonal pattern of boundary defecations (above) and hinterland defecations (below) by different age and sex classes and by the groups; figures for individual age and sex classes are the mean number per badger per night ± s.e and for the groups are the mean number per group per night ± s.e.

	Spring	Summer	Autumn	Winter
Adult males	0.78 ± 0.34	0.50 ± 0.71	0.50 ± 0.71	0.33 ± 0.41
	0.28 ± 0.20	0.50 ± 0.71	0	0
Adult females	0.14 ± 0.15	0.75 ± 0.55	0.05 ± 0.06	0.17 ± 0.18
	0.71 ± 0.20	0.13 ± 0.14	0.45 ± 0.26	0.58 ± 0.22
Yearling males	0.67 ± 0.41	0.50 ± 0.71	0.50 ± 0.71	0
	0.17 ± 0.20	0.33 ± 0.41	0.50 ± 0.71	0
Yearling females	0.50 ± 0.33	—	—	—
	0.25 ± 0.29	—	—	—
Male cubs	—	0	0.25 ± 0.29	0.17 ± 0.20
	—	0	0.50 ± 0.33	0.50 ± 0.35
Female cubs	—	0	0	0
	—	0.25 ± 0.29	0.88 ± 0.36	0.75 ± 0.55
Groups	2.68 ± 0.50	5.00 ± 1.00	2.39 ± 0.45	1.72 ± 0.47
	4.02 ± 0.92	2.85 ± 0.42	8.33 ± 1.16	7.58 ± 1.21

visited significantly more boundary latrines in the winter than the other categories (one-way ANOVA, $F_{(category)}=4.40$, d.f.$=4,15$, $p<0.05$, Tukey's HSD) (Table 3.3).

Hinterland latrine visits by adult males reached a peak in the summer (Table 3.3). During this season adult males visited significantly more hinterland latrines per night than the cubs (one-way ANOVA, $F_{(category)}=5.29$, d.f.$=3,13$, $p<0.05$, Tukey's HSD). For individual age and sex categories and the group category no significant seasonal trend was detected in the number of latrines (boundary and/or hinterland) visited per night.

3.3.5 Seasonal pattern of anal gland secretions

Production of anal gland secretion reached a peak in the winter (Table 3.4), with the mean number per group per night significantly greater than in the summer (Kruskal-Wallis, $H=15.57$, d.f.$=3$, $p=0.001$). No seasonal trends for individual age and sex categories were detected; in all cases Kruskal-Wallis, $p>0.5$. During the year females of all ages produced significantly more anal gland secretion than males (Kruskal-Wallis, $H=4.90$, d.f.$=1$, $p<0.05$). During the combined winter and spring, adult females deposited significantly more anal gland secretion at hinterland than boundary latrines (Kruskal-Wallis, $H=4.29$, d.f.$=1$, $p<0.05$), but they showed no significant preference for the type of latrine used for their anal gland secretions in the combined summer and autumn period.

3.3.6 Seasonal pattern of movement within the territory

The nightly distance travelled within 100m of the main sett reached a peak in adult females in spring (Table 3.5), but this was not significantly different from the other categories (one-way ANOVA, $F_{(category)}= 2.92$, d.f.$=4,15$, $p>0.05$).

social group. Knowledge of the location of the boundary path that connected the peripheral latrines of a group, combined with the bait marking, enabled latrines to be differentiated into boundary and hinterland latrines. For these five study groups mean territory size in 1991 was 37.2 $\pm$ 7.1 ha, mean territorial boundary length 2.71 $\pm$ 0.23 km, mean group size 17.0 $\pm$ 2.8 badgers, and mean number of setts per social group (excluding the main sett) 5.0 $\pm$ 0.6. Within these territories mean area of woodland was 6.24 $\pm$ 2.38 ha and mean area of permanent pasture 25.36 $\pm$ 2.86 ha.

1.5 The thesis

1.5.1 Thesis structure

Following the introduction, Chapter 2 describes how spool-and-line tracking in conjunction with a biomarker was used to follow the detailed movement patterns of individual animals and together enabled badger excretory products to be located. Construction of the spool-and-line and the problems associated with spool-and-line tracking such as tracking animals the night after capture are included, together with the protocol used to ensure the minimum impact on the animals behaviour the night following capture. Chapter 3 examines how these techniques were implemented to analyse the role of the individual within the social group in scent marking and territorial behaviour. Scent marking and spatial data from different age and sex categories were used to compare the food-based and sex-based hypotheses which attempt to explain the evolution of group living in the badger. Chapter 4 examines the significance of scent marking by badgers in the transmission of bovine tuberculosis from badgers to cattle. Data were collected using the combination of spool-and-line tracking and biomarker and were analysed at the group rather than the individual level. Analyses included the seasonal pattern of excretory behaviour

Table 3.4 Seasonal pattern of anal gland secretions by different age and sex classes and by the groups; figures for individual age and sex classes are the mean number per badger per night ± s.e. and for the groups are the mean number per group per night ± s.e.

	Spring	Summer	Autumn	Winter
Adult males	0	0	0	0.17 ± 0.20
Adult females	0.57 ± 0.32	0.13 ± 0.13	0.30 ± 0.22	0.58 ± 0.34
Yearling males	0	0	0	0
Yearling females	0.50 ± 0.29	—	—	—
Male cubs	—	0	0	0
Female cubs	—	0	0	0.50 ± 0.58
Groups	2.74 ± 0.68	0.62 ± 0.16	1.44 ± 0.36	5.19 ± 0.90

Table 3.5 Distance travelled within 100m of the main sett by different age and sex classes; figures are the mean distance travelled per badger per night in m ± s.e. (above) and the mean proportion ± s.e. of the total distance travelled per badger per night (below).

	Spring	Summer	Autumn	Winter
Adult males (old)	303 ± 91	246 ± 194	86 ± 86	204 ± 33
	0.21 ± 0.08	0.11 ± 0.04	0.03 ± 0.04	0.18 ± 0.05
Adult males (young)	133 ± 53	—	—	—
	0.15 ± 0.06	—	—	—
Adult females	463 ± 106	399 ± 86	165 ± 52	263 ± 50
	0.32 ± 0.07	0.19 ± 0.04	0.46 ± 0.18	0.43 ± 0.14
Yearling males	201 ± 72	196 ± 53	197 ± 8	288 ± 60
	0.19 ± 0.05	0.14 ± 0.03	0.16 ± 0.02	0.27 ± 0.06
Yearling females	186 ± 51	—	—	—
	0.18 ± 0.07	—	—	—
Male cubs	—	420 ± 37	247 ± 63	141 ± 54
	—	0.36 ± 0.03	0.18 ± 0.05	0.27 ± 0.12
Female cubs	—	152 ± 27	331 ± 84	312 ± 108
	—	0.15 ± 0.03	0.18 ± 0.05	0.29 ± 0.07

During the summer, male cubs travelled a significantly greater distance around the main sett than female cubs (one-way ANOVA, $F_{(category)}=4.27$, d.f.=4,12, $p<0.05$, Tukey's HSD) and a greater proportion of the total distance travelled was around the main sett than for all other age and sex categories (one-way ANOVA, $F_{(category)}=9.74$, d.f.=4,12, $p<0.001$, Tukey's HSD). A significant seasonal variation was also detected in male cubs. The distance travelled within 100m of the main sett was at a maximum in the summer, and declined with age, being significantly less in the winter (one-way ANOVA, $F_{(season)}=9.43$, d.f.=2,8, $p<0.01$, Tukey's HSD). For adult males, a greater proportion of the total distance travelled was around the main sett in the winter/spring than in the summer/autumn (two-way ANOVA, $F_{(season)}=7.29$, d.f.=1,5, $p<0.05$).

When looking at activity within 50m of the territory boundary, adult males travelled a significantly greater distance on the boundary than all other age and sex categories in the winter (one-way ANOVA, $F_{(category)}=9.47$, d.f.=4,15, $p<0.001$, Tukey's HSD) (Table 3.6). The only other significant seasonal trend was for male cubs, which in the autumn travelled significantly further on the territorial boundary than in summer or winter (one-way ANOVA, $F_{(season)}=9.89$, d.f.=2,8, $p<0.01$, Tukey's HSD). When the data were reanalysed to allow for differences between groups in the length of the territorial boundary (Table 3.7), adult males travelled a significantly greater proportion of the territory boundary in the autumn than all other categories (one-way ANOVA, $F_{(category)}=3.39$, d.f.=4,12, $p<0.05$, Tukey's HSD). Adult males also covered a significantly greater proportion of the territory boundary than yearling males and female cubs in the winter (one-way ANOVA, $F_{(category)}=3.52$, d.f.=4,15, $p<0.05$, Tukey's HSD).

Table 3.6 Distance travelled within 50m of the territory boundary by different age and sex classes; figures are the mean distance travelled per badger per night in m ± s.e. (above) and the mean proportion ± s.e. of the total distance travelled per badger per night (below).

	Spring	Summer	Autumn	Winter
Adult males (old)	792 ± 365	865 ± 670	1485 ± 1245	303±65
	0.46 ± 0.09	0.40 ± 0.15	0.58 ± 0.39	0.25 ± 0.07
Adult males (young)	106 ± 38	—	—	—
	0.11 ± 0.04	—	—	—
Adult females	303 ± 181	591 ± 82	129 ± 90	44 ± 31
	0.15 ± 0.09	0.28 ± 0.05	0.15 ± 0.08	0.06 ± 0.05
Yearling males	247 ± 128	580 ± 331	320 ± 149	96 ± 56
	0.26 ± 0.15	0.35 ± 0.17	0.25 ± 0.08	0.09 ± 0.04
Yearling females	349 ± 257	—	—	—
	0.21 ± 0.14	—	—	—
Male cubs	—	173 ± 12	470 ± 118	59 ± 36
	—	0.15 ± 0.01	0.31 ± 0.05	0.14 ± 0.10
Female cubs	—	167 ± 25	440 ± 104	0
	—	0.17 ± 0.02	0.23 ± 0.04	—

Table 3.7 Seasonal pattern of distance travelled along the territorial boundary path by different age and sex classes; the figures are mean percent of total boundary length ± s.e. travelled per badger per night.

	Spring	Summer	Autumn	Winter
Adult males (old)	10.7 ± 4.6	22.7 ± 22.5	46.0 ± 38.1	5.9 ± 3.7
Adult males (young)	0.7 ± 0.4	—	—	—
Adult females	8.8 ± 5.7	5.2 ± 2.0	3.3 ± 2.0	1.0 ± 1.1
Yearling males	7.5 ± 6.0	6.2 ± 5.0	12.1 ± 7.2	0.2 ± 0.2
Yearling females	5.7 ± 4.1	—	—	—
Male cubs	—	0	2.0 ± 1.8	0.9 ± 0.6
Female cubs	—	0	0	0

3.3.7 Seasonal pattern of sett visits

Excluding the main sett, both male and female cubs entered significantly more setts during the night than all other age and sex categories in the summer (Kruskal-Wallis, $H=10.62$, d.f. $=4$, $p<0.05$) and autumn (Kruskal-Wallis, $H=11.29$, d.f. $=4$, $p<0.05$) (Table 3.8). Badger cubs (sexes combined) also visited, without entering, significantly more setts in the autumn than adults and yearlings (sexes combined) (Kruskal-Wallis, $H=3.83$, d.f. $=1$, $p<0.05$). Adult males visited, without entering, significantly more setts during the winter/spring than the summer/autumn (Kruskal-Wallis, $H=4.34$, d.f. $=1$, $p<0.05$).

3.3.8 Seasonal pattern of extra-territorial forays

At no stage were females observed to cross a territory boundary and temporarily leave their natal territory. This behaviour was only recorded in adult and yearling males (Table 3.9). The area of overlap of nightly minimum convex polygon ranges into neighbouring territories by adults in their first spring was significantly greater than the other age and sex categories (Kruskal-Wallis, $H=13.31$, d.f. $=4$, $p<0.01$). No other seasonal or age and sex related trends were detected; in all cases Kruskal-Wallis $p>0.5$.

3.3.9 Pattern of scent marking by cubs

No urinations were recorded from all eight badger cubs tracked during the summer, and in the autumn cubs of either sex urinated above ground infrequently (mean 0.38 ± 0.20 urinations per night). When they did, the urine was deposited randomly and not located at a latrine or in association with a badger run (Figure 3.2). During the winter badger cubs again urinated infrequently (mean 0.29 ± 0.20

Table 3.8 Seasonal pattern of visits to setts other than the main sett, showing setts entered (above) and not entered (below) by different age and sex categories; figures are the mean number of different setts visited per badger per night ± s.e.

	Spring	Summer	Autumn	Winter
Adult males	0.25 ± 0.17	0.50 ± 0.71	0	0.17 ± 0.20
	1.00 ± 0.49	0	0	0.83 ± 0.54
Adult females	0.14 ± 0.15	0.25 ± 0.29	0.15 ± 0.17	0.33 ± 0.23
	0.57 ± 0.32	0.75 ± 0.29	0.20 ± 0.22	0.42 ± 0.22
Yearling males	0.17 ± 0.20	0.33 ± 0.41	0	0.25 ± 0.29
	0.83 ± 0.20	0.67 ± 0.41	0.50 ± 0.71	0.75 ± 0.87
Yearling females	1.00 ± 0.47	—	—	—
	0.50 ± 0.33	—	—	—
Male cubs	—	1.50 ± 0.33	1.00 ± 0.47	1.00 ± 0.71
	—	0.38 ± 0.28	0.75 ± 0.29	0.33 ± 0.41
Female cubs	—	2.00 ± 0.47	1.38 ± 0.28	0.75 ± 0.55
	—	0	0.63 ± 0.28	0.75 ± 0.29

Table 3.9 Seasonal pattern of nightly minimum convex polygon range overlap into neighbouring territories by different age and sex classes; figures are the mean areas of overlap (ha) per badger per night $\pm$ s.e.

	Spring	Summer	Autumn	Winter
Adult males (old)	0	3.25 $\pm$ 4.59	1.01 $\pm$ 1.43	1.60 $\pm$ 1.96
Adult males (young)	2.52 $\pm$ 0.24	—	—	—
Adult females	0	0	0	0
Yearling males	0	1.42 $\pm$ 1.78	0	0.55 $\pm$ 0.45
Yearling females	0	—	—	—
Male cubs	—	0	0	0
Female cubs	—	0	0	0

Figure 3.2 Spool-and-line trace for one night for (a) a female cub in July, and (b) a female cub in October from the same social group. Legend as in Figure 3.1.

(a)

(b)

urinations per night), but for the first time latrines were used to deposit urine, which was no longer deposited randomly.

Badger cubs defecated infrequently during the summer (mean 0.13 $\pm$ 0.13 defecations per night). By the autumn and winter badger cubs were depositing as many faeces above ground as the other age and sex categories (mean 0.81 $\pm$ 0.20 and 0.71 $\pm$ 0.29 defecations per night) respectively. During these three seasons faeces were always deposited at latrines, with male cubs alone depositing a proportion at boundary latrines (Table 3.2).

Correlated with the low levels of above-ground urinating and defecating behaviour of young badgers, there were low levels of bite wounding, and these rose as the cubs started to excrete above ground and explore the range more extensively (Table 3.10). When the bite wound categories and sexes were combined, significantly fewer badger cubs had bite wounds in the summer than cubs in the autumn and winter, and yearlings in the spring (X^2=26.94, df=3, $p<0.001$).

3.4 Discussion

Previous work on badger scent marking has concentrated on the role of faeces as a territorial marker, with some work on anal secretion (Davies, Lachno & Roper 1988) and sub-caudal glands (Gorman, Kruuk & Leitch 1984; Kruuk, Gorman & Leitch 1984). The importance of urine as a territorial marker has so far been ignored because of the difficulty of locating urine in the field. Yet the present study found that there were no significant differences in the number of defecations per night between the various age and sex classes, nor were there any seasonal trends in faecal production by any of the age and sex categories. Urine scent marks were much more variable and clearly the use of urine as a territorial marker in badgers has hitherto been under-rated.

Table 3.10 Pattern of bite wounding in young badgers; unpublished data from W.J. Cresswell and S. Harris.

	Males			Females		
	None	Minor wounds	Moderate/ extensive wounds	None	Minor wounds	Moderate/ extensive wounds
Cubs						
Summer	30	2	1	27	2	0
Autumn	7	4	1	10	2	0
Winter	4	4	2	10	3	0
Yearlings						
Spring	14	7	1	13	5	0

If territorial behaviour in badgers evolved to restrict access to oestrous sows, there should be seasonal differences in urinating and/or dunging behaviour between the sexes in adult badgers, and a seasonality synchronized with the breeding season. Roper, Shepherdson & Davies (1986) argued that the pattern of territorial scent marking with faeces and anal gland secretions is bimodal, with a large spring peak which they suggested is associated with the spring mating period, and an autumn peak believed to be due to an upsurge in mating behaviour at that time. Since the winter/spring mating period is over twice as important in contributing to overall fecundity (Cresswell *et al.* 1992), if latrine use is associated with reproductive activity the pattern of latrine use should reflect the much greater importance of the winter/spring mating period.

However Roper, Shepherdson & Davies (1986) reported that the spring and autumn peaks were very similar. Also latrine use reported by Roper, Shepherdson & Davies (1986) was at its greatest in April and October, whereas the peak in pre-ovulatory follicles in adult sows occurs in January and August (Cresswell *et al.* 1992). Thus the peaks in latrine use reported by Roper, Shepherdson & Davies (1986) occur after the main mating periods, not just prior to or during as might be expected. Finally, the present study showed that there was no significant seasonal variation in faeces production by any age and sex category, although mean group daily above ground faecal production did show seasonal variations due to changes in group composition. Thus for each social group, mean daily above ground faecal production (excluding yearling sows, for which there were inadequate data) was lowest in spring (6.73) before the cubs emerged, rising to 8.05, 10.70 and 10.11 in the summer, autumn and winter respectively. Thus, it would appear that the low number of faeces at latrines in summer as reported by Roper, Shepherdson & Davies (1986) probably represents in large part seasonal differences in faecal decomposition rates rather than any behavioural differences. Although these figures appear very low it is important to stress that these figures and in fact all figures on

the nightly production of excreta represent above ground production only. It is possible that since badgers were only spending about 2.5 hours active above ground per night throughout the year (see Chapter 2, Figure 2.3), large quantities of excreta may have been deposited below ground, which were not recorded in this study.

Perhaps a better measure of a badger's territorial behaviour would be to examine seasonality of anal gland secretions and patterns of range and latrine utilization. Roper, Shepherdson & Davies (1986) defined a second type of latrine called a temporary defecation site. These sites usually only contained a single dung pit and were used infrequently, with faeces found during only one or two months of the year. These authors found a peak in anal gland secretions at latrines in the spring and temporary defecation sites in the winter, and suggested that anal gland secretions were deposited around territory boundaries for the purpose of territorial defence. Their timing of the peak of anal gland secretions is consistent with the present study; however, its role as a territorial marker is arguable. Anal gland secretions were produced almost exclusively by adult females and deposited primarily at hinterland latrines. Contrary to the views of Davies, Lachno & Roper (1988), Gorman, Kruuk & Leitch (1984) suggested that badger anal gland secretion is highly volatile and might therefore be used for short term communication; it would not therefore be a particularly effective boundary scent marker. The view of Gorman, Kruuk & Leitch (1984) is supported by the timing of anal gland secretions, and the fact that they are produced mainly by females suggests that these may convey information on sexual condition. Consistent with this idea is the fact that adult males visited a greater proportion of hinterland latrines at this time of year than at any other stage and in the winter/spring travelled a greater distance in the vicinity of the main sett and visited more setts. This behaviour could imply some degree of mate guarding by adult males, even though adult badgers were rarely seen to travel together. Activity around setts may be a more effective

strategy than boundary patrolling/scent marking, given the distance travelled in the region of the main sett by adult females, which reached a peak in the spring. Since most recorded matings take place on the main sett and given that intruding males headed straight for main setts, resident boars may be showing mate guarding, although at this stage there is only weak evidence to support this.

So why should a female in oestrus need to advertise her condition to the resident boars, since occupation of the same sett would enable the boar(s) to detect the odour of an oestrous female immediately? Whilst most adult males occupy the main sett (Roper & Christian 1992; J. Brown unpublished), younger females with cubs often occupy annexe setts during the mating period to avoid the aggression of older sows (Cresswell *et al.* 1992), so at least some breeding sows may need to advertise their condition to the resident boars. With a mean group size of 17 for this study, a greater proportion of animals may spend the day away from the main sett, thereby enhancing the need for advertisement. Also, the few observations on mating in wild badgers suggest that several different boars may mate with a sow during one oestrus (Neal & Harrison 1958; Paget & Middleton 1974). Hence an oestrous sow may need to advertise her condition to all the males within the group, some of whom may be occupying outlying setts (Kruuk 1978) to avoid dominant males, since aggression is greatest at this time of year (Cresswell *et al.* 1992). Finally, a sow would need to advertise her sexual condition to ingressing males from neighbouring groups, and since cuckoldry is the main means of gene flow (Evans, Macdonald & Cheeseman 1989), this may be even more important than advertising to males of the same group. In the spring, intruding males visited and sometimes even remained in the main sett during the day, and this was the area of greatest activity and marking with anal secretions by the adult sows.

The greater extent of male extra-territorial movements has been reported previously (Cheeseman *et al.* 1988). The significantly greater area of overlap by young adult males into neighbouring territories during the spring suggests that these

animals were in search of oestrous females. All of the young adult males performing these extra-territorial movements visited the neighbouring group's main sett, presumably since adult sows concentrated their activity around the main sett in the spring. As badgers become sexually mature between one and two years of age (Ahnlund 1980), these observations suggest that younger males may be prevented access to oestrous sows within their natal group by dominant boar(s), which have some degree of preferential access to oestrous sows.

Although not significant, the number of boundary latrines visited by adult males and boundary urinations reached a maximum in the summer and autumn, and hinterland urinations were most frequent in the summer. Above-ground activity by all badgers is reduced in the winter (Harris 1982), and the fact that adult males urinated significantly more at boundary latrines in winter than other age and sex categories was due to a decrease in boundary activity by other animals and not an increase by adult males. The summer and autumn peak in territorial behaviour seen in adult males clearly does not correspond with the predictions of the sex hypothesis and may be more easily explained by the food requirements of badgers in the summer and autumn. Food is probably limiting on the study area; Cheeseman *et al.* (1993) found a significant increase in cub growth rates and adult body weights following badger removals. The food hypothesis predicts that territorial behaviour will reach a peak when food availability is at its lowest. Earthworms are most active on the surface in spring and autumn (Evans & Guild 1947), but become inactive during periods of dry weather in the summer. Other food sources such as fruits and seeds are also unavailable at this time of year, suggesting that in summer food availability to badgers can be low. In the autumn, whilst food resources are more abundant, group energy demands are greater due to the increased size of the cubs, and the need to lay down fat for the winter. Breeding females in particular show greatest annual variation in body weight and lower survival rates than non-

breeding females (Cheeseman *et al.* 1987), suggesting that adequate food reserves in the autumn are important for over-winter survival.

Also in agreement with the food hypothesis was the observation that all adult and yearling categories exhibited territorial behaviour. Food-based models of territoriality predict that all members of the social group take part (Macdonald 1983), and that territoriality should be most marked in females, since female reproductive success is food-limited (Trivers 1972; Wrangham 1982). During the summer, however, it was adult males that urinated significantly more than other animals, and visited significantly more latrines than other age and sex groups, except yearling males. Although the number of boundary urinations by adult females also reached a peak in this season, it was not significantly different from other age and sex categories, and adult females showed no significant seasonal variation in boundary urinations. The mean number of all urinations per night by adult females was also maximum during summer, but again showed no significant seasonal variation. Finally, all animals combined show greatest boundary activity in the summer and autumn, with this being greatest in the summer for adult sows and yearling males, and in the autumn for adult males and cubs.

Roper, Shepherdson & Davies (1986) suggested that as cubs grow and reach adult size the demand for food within the group increases from spring to autumn, and as food availability is probably at its lowest in the summer, competition for food is probably greatest during June and July. Therefore there may be strong pressures on cubs, particularly in summer, to maintain a low profile to prevent conflict and possible expulsion from the group. These predictions are supported by the clandestine behaviour of cubs. The total lack of urine above ground during the summer and the random nature of its distribution on pasture in the autumn suggests that cubs are trying to conceal this particular scent marker from other members of the social group. During summer/autumn the few cub defecations that occurred above ground were always situated at latrines. Thus although urine marks were

being concealed, at least some of the faeces were deposited at latrines where they would be detected by other members of the social group. It is possible, however, that the faeces do not provide information on a badger's individual identity, just group identity. Davies, Lachno & Roper (1988) found no evidence that badger anal gland secretion aided individual identity, but did aid group identity.

Cubs also spent a large proportion of the night below ground at setts other than the main sett, which they frequently visited during the course of the night. It is probable that these setts were used for urinating and defecating, since latrines have been found in a number of excavated setts (Roper 1992; S. Harris unpublished). Since cubs were never observed foraging with their mother from June onwards, cubs may also avoid the aggression of other group members by remaining in these setts for long periods. The presence of annexe setts has been shown to correlate with increased reproductive success of younger sows, and this is probably due to reduced levels of infanticide (Cresswell *et al.* 1992). Direct evidence for infanticide by dominant sows is lacking, and the few recorded cases have been of young cubs (Lüps & Roper 1990; S Harris unpublished). However, it is possible that cubs are still vulnerable to aggression from other sows during the summer and even the autumn. Hence their reduced activity levels above ground, the restriction of their activity to the area around setts, and frequent visits to setts which provide some protection. Associated with this clandestine behaviour were very low levels of bite wounding in cubs compared to older animals (Cresswell *et al.* 1992).

In conclusion, a detailed study of individual badgers has shown that territorial behaviour is more complex than previously described. Most of the predictions of the hypothesis that group living in badgers evolved to monopolise access to oestrous females are not borne out. Whilst adult males urinated more on boundaries in the winter, visited more latrines in the winter, and perhaps move more on the boundaries in the winter than other age and sex categories, this is a

period of very reduced activity by badgers generally, and not the period of greatest territorial incursions by neighbouring males. Not surprisingly, therefore, whilst boundary activity in the winter was significantly higher for adult males than other age and sex categories, it was a period of reduced boundary patrolling and relatively low urine marking and latrine visits by adult males compared to other seasons. Although the few observations of matings suggest that individual boars do not monopolise access to oestrous sows, the increased extra-territorial movements of younger males in the spring suggests that dominant boars do have some preferential access to oestrous sows.

In contrast, more of the predictions of the food hypothesis are borne out. Thus most territorial activity occurs in the summer and autumn, and in the autumn adult males travel greater distances boundary patrolling, have more boundary urinations, and visit the greatest number of latrines. Contrary to the predictions of the food based hypothesis, whilst all age and sex categories play a role in territorial demarcation in the summer and autumn, this was not most pronounced in females, and in fact for adult females latrine visits were greatest in the spring, declining in the summer and autumn. Also as predicted, young cubs do not advertise their presence to other group members during times of greatest food shortage and spend a greater amount of time in or near a sett. Associated with this clandestine behaviour are low levels of bite wounding in cubs. Thus whilst the hypotheses are not mutually exclusive (Cresswell *et al.* 1992) these observations favour the food-based as opposed to the sex-based hypothesis as the major factor leading to the evolution of group living and territorial behaviour in badgers.

Recently another hypothesis attempting to explain territoriality has been put forward by Doncaster & Woodroffe (1993). They suggested that territoriality in badgers functions to defend an established breeding site, thereby maximising long-term reproductive success. For a Bristol population of badgers, Cresswell & Harris (1988) found that latrines were clumped around setts, with latrines most clumped

around setts in the spring, whereas in the autumn they were more dispersed. This is consistent with this hypothesis as defence of setts is likely to reach a peak during the breeding season. As the general pattern is for animals to breed and overwinter in the main sett (Roper 1992), it should be expected that defence of the main sett will be greater than for other setts. However, Cresswell & Harris (1988) found that the distribution of latrines in relation to the nearest main sett was more random compared to the distribution to the nearest sett. These authors also reported no change in the number of faeces per latrine with increasing distance from the sett. Clearly this hypothesis is difficult to test directly with the data presented in this chapter. It would also be particularly difficult to distinguish between scent marking and spatial behaviour involved in defending a breeding sett with behaviour involved in defending a breeding sow.

As well as evaluating the role of the individual in scent marking and territoriality, the spool-and-line and biomarker technique has enabled the seasonal pattern of distribution of badger excreta to be examined. The applied aspects of badger excretory behaviour and in particular the seasonal contamination of different habitats and the identification of excretion sites are discussed in the next chapter.

CHAPTER 4

BADGER EXCRETORY BEHAVIOUR AND THE TRANSMISSION OF TB TO CATTLE

4.1 Introduction

The situations in which cattle make contact with (i.e. investigate and/or consume) badger excretory products remains unknown. Cattle have been shown to strongly avoid the ingestion of badger urine and faeces (Benham & Broom 1991) This observation led these authors to conclude that cattle were unlikely to contract tuberculosis by the ingestion of contaminated pasture and since the majority of cattle totally avoided excreta, the inhalation of bacilli was also unlikely. However, Benham & Broom (1991) also found that a small proportion of cattle were totally unselective towards badger excreta with the extent of this unselectivity increasing as the amount of attractive herbage available to the cattle decreased. In other recent studies, cattle have shown a positive preference for grazing on simulated cattle urine patches because of the higher nitrogen content of the vegetation in these patches (Jaramillo & Detling 1992). Whether badger urine is also attractive for the same reason is unknown.

The dose of tubercle bacilli required to produce infection via the alimentary route is several thousand times greater than the dose required to produce infection via the respiratory route (Francis 1958). Francis (1971) examined the route of infection in 56,000 cattle from several countries and found that the lungs were infected about ten times more frequently than the abdominal cavity, indicating the route of infection to be aerogenous in 90% of cases. Infection of the lungs is also possible via an indirect route from the rumen. Waldo & Hoernicke (1961) found

large numbers of marker bacteria in the lungs after being eructated in gases from the gut. Lung infections could therefore result from either direct inhalation of aerosols from infected excreta or alternatively by cattle grazing contaminated pasture.

When one or more cattle in a herd reacts positively to the tuberculin test, there is a detailed investigation of all the possible sources of infection by the Ministry of Agriculture, Fisheries and Food. Where badgers are considered to be the most likely source of infection a badger control operation is usually instigated. Despite the various control operations, the number of herds with reactor cattle has remained higher in the south-west than the rest of Britain, and there has been no significant decline in the bovine tuberculosis problem (Ministry of Agriculture, Fisheries and Food 1993). In the south-west bovine tuberculosis is still largely confined to the same limited areas (about 12% of the total land area) of south-west England.

The current advice supplied by the Ministry of Agriculture, Fisheries and Food to farmers is to exclude cattle from badger setts and badger latrines on pasture. The former is due to the head rubbing behaviour of cattle on badger setts and because cattle may investigate discarded bedding material at sett entrances. The fencing of badger latrines would minimise contact between cattle and badger excreta located at these sites. To avoid the contamination of cattle food, the Ministry advise the feeding of cattle at pasture with 'badger-proof' troughs and deny access to badgers of farm buildings containing food stores.

In this chapter I will concentrate on the applied aspects of badger excretory behaviour by examining how this behaviour may be involved in the transmission of bovine tuberculosis from badgers to cattle. Analyses will include the seasonal pattern of contamination of different habitat types with badger urine and faeces and the seasonal distribution of excreta on badger territories. With an improved understanding of the factors affecting the deposition of faeces and urine by badgers

on their territories, it should be possible to identify ways of reducing contact between cattle and badger excretory products, and hence ultimately reduce the spread of bovine tuberculosis from badgers to cattle.

4.2 Methods

For each night's data the number of latrines visited, number of urinations, defecations and anal gland secretions were recorded. Sites of excretion were marked on location with conspicuous markers enabling their exact positioning on a map of scale 1:2500. Movements were also recorded on the same scale map for subsequent analysis of the pattern of range use. The following data were recorded from each latrine visited by a focal animal; number of fresh faeces (judged to be less than three days old), number of old faeces, number of fresh pits (as for faeces), number of old pits, number of faeces in and out of pits, number of anal gland secretions associated with faeces and number alone and a score (0-5) of the extent of scraping. Although the ageing of faeces and pits was relatively subjective, the accuracy of age assessment was increased by comparing the decomposition of faeces and deterioration of pits with faeces and pits of known age produced by spool-and-line tracked animals.

4.3 Results

4.3.1 Seasonal pattern of urine distribution on badger territories

This analysis examined the seasonal distribution of urine between those sites most regularly used for urinating (latrines, *runs and random*), across all habitat types (woodland, pasture and arable land). The number of urinations in the spring season on runs and latrines was significantly greater than the number of random

urinations (Friedman's analysis of variance, $F=15.27$, d.f. $=2,38$, $p=0.0001$ Tukey's HSD) (Table 4.1). This pattern was repeated in the summer (Friedman's, $F=5.95$, d.f. $=2,32$, $p<0.01$, Tukey's HSD) and winter (Friedman's, $F=3.72$, d.f. $=2,38$, $p<0.05$, Tukey's HSD). However in the autumn season no significant difference in the general distribution of urine between these three sites was detected (Friedmans, $F=0.58$, d.f. $=2,32$, $p>0.05$).

4.3.2 Seasonal pattern of urination beside field boundaries

The mean number and mean proportion of urinations on runs crossing field boundaries (crossing point runs) per night on all habitat types reached a peak in the summer season (Table 4.2). Although there was an overall significant difference (Kruskal-Wallis, $H=8.37$, d.f. $=3$, $p<0.05$) in the mean number of urinations, the conservative nature of the Tukey-type multiple comparison of means meant that no single season could be isolated as being statistically distinct from any other. No significant seasonal variation in the mean proportion of urinations on crossing point runs was detected ($H=6.87$, d.f. $=3$, $p>0.05$).

A peak in the mean number and mean proportion of urinations at crossing point runs on pasture occurred in the spring. As with the above, an overall significant difference in the mean number of urinations was detected ($H=8.14$, d.f. $=3$, $p<0.05$), but no single season could be isolated as being statistically distinct. Again mean proportion of urinations at these sites showed no significant seasonal variation ($H=6.33$, d.f. $=3$, $p>0.05$).

Urinations on these runs either took the form of trails (mean proportion 0.61 $\pm$ 0.09) or patches (mean proportion 0.39 $\pm$ 0.09). At crossing point runs on pasture, trails measured up to 1.60 m in length (mean 0.75 $\pm$ 0.12 m), with a mean patch diameter of 0.13 $\pm$ 0.01 m. They were normally deposited on the badger path just after the badger had passed through the linear feature, but occasionally

Table 4.1 Seasonal pattern of urine distribution; figures are the mean number per badger per night ± s.e. (above) and the mean proportion of total urinations per badger per night ± s.e. (below).

	Spring	Summer	Autumn	Winter
Latrine	1.16 ± 0.31	0.88 ± 0.43	0.84 ± 0.55	0.38 ± 0.15
	0.61 ± 0.09	0.48 ± 0.14	0.46 ± 0.16	0.50 ± 0.16
Run	0.70 ± 0.17	0.91 ± 0.38	0.24 ± 0.11	0.63 ± 0.29
	0.38 ± 0.08	0.50 ± 0.14	0.24 ± 0.13	0.50 ± 0.16
Random	0.05 ± 0.05	0.06 ± 0.06	0.18 ± 0.10	0
	0.01 ± 0.01	0.02 ± 0.02	0.30 ± 0.15	0

Table 4.2 Seasonal pattern of urination on crossing point runs (all habitat types) and on pasture alone; figures are the mean number of urinations per badger per night ± s.e. (above) and mean proportion of total urinations per badger per night ± s.e. (below).

	Spring	Summer	Autumn	Winter
Habitats grouped	0.63 ± 0.17	0.71 ± 0.34	0.12 ± 0.08	0.35 ± 0.19
	0.31 ± 0.08	0.34 ± 0.12	0.04 ± 0.04	0.28 ± 0.13
Pasture	0.53 ± 0.16	0.44 ± 0.25	0.12 ± 0.08	0.23 ± 0.16
	0.26 ± 0.08	0.23 ± 0.09	0.04 ± 0.04	0.24 ± 0.13

when it was travelling parallel to the linear feature. Although over 90% of the crossing point urinations on pasture were within 4 m of the linear feature, a small proportion were deposited up to 7.5 m away (Figure 4.1).

The proportions of crossing point urinations at different types of field boundary are shown in Figure 4.2. Badgers urinated more than expected at crossing points on boundaries with restricted access (sheep netting, thick undergrowth and hedges), and less than expected on boundaries with less restricted access (open woodland, barbed wire and walls with gates) ($X^2=6.61$, d.f.$=1$, $p<0.05$).

The number of crossing point urinations was significantly correlated with the number of boundaries crossed per badger per night per km travelled both for all habitat types and for pasture alone (Figure 4.3); thus there was an increase in the number of crossing point urinations with the number of boundaries crossed. Taking account of the mean group size for the five study groups, and using the mean number of urinations for each age and sex category, the projected number of crossing point urinations on pasture per night per group is 6.2 in spring, 7.7 in summer, 1.5 in autumn and 2.4 in winter.

4.3.3 Seasonal pattern of latrine use on pasture

No significant seasonal variation was detected in the mean number of urinations per night deposited at latrines on pasture (Kruskal-Wallis, H=4.10, d.f.$=3$, $p>0.05$), nor the mean proportion of urinations at pasture latrines (H=3.32, d.f.$=3$, $p>0.05$) (Table 4.3). For defecations at pasture latrines a significant seasonal trend was detected, with the mean number of defecations per night during the autumn being significantly greater than in the winter season (H=9.02, d.f.$=3$, $p<0.05$). This pattern was repeated for the mean proportion of defecations per night at latrines on pasture, although latrines on pasture during the

Table 4.3 Seasonal pattern of urination and defecation at latrines on pasture; figures are the mean number per badger per night ± s.e. (above) and mean proportion of total urinations/defecations per badger per night ± s.e. (below).

	Spring	Summer	Autumn	Winter
Urine	0.45 ± 0.31	0.74 ± 0.43	0.66 ± 0.54	0.05 ± 0.05
	0.17 ± 0.09	0.41 ± 0.15	0.27 ± 0.13	0.09 ± 0.10
Faeces	0.35 ± 0.11	0.35 ± 0.15	0.50 ± 0.13	0
	0.37 ± 0.12	0.59 ± 0.19	0.73 ± 0.13	-

Figure 4.1 Cumulative percentage of distances of crossing point urinations from field boundaries; the figure shows the furthest point of the trail or patch of urine from the field boundary.

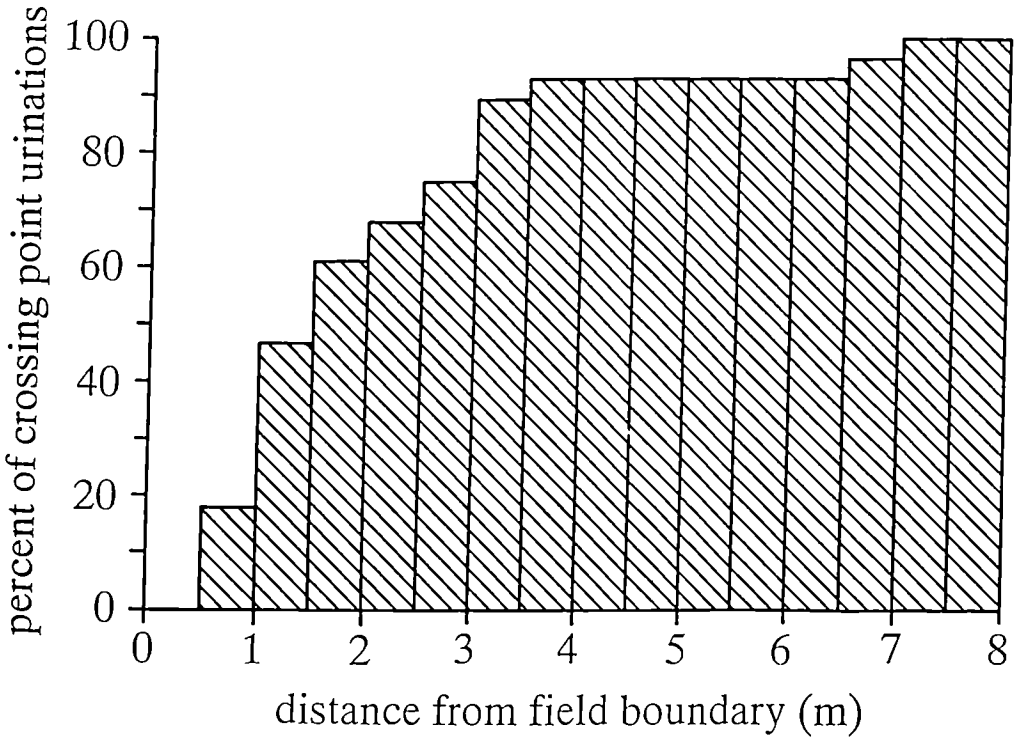


Figure 4.2 Proportion of different types of field boundaries selected for depositing urine on crossing point runs; combined hedges are those in conjunction with either barbed wire, sheep netting or a wall. The proportion of field boundaries selected was calculated by dividing the mean number of crossing point urinations night^{-1} boundary type^{-1} by the mean number of crossings night^{-1} of that boundary type.

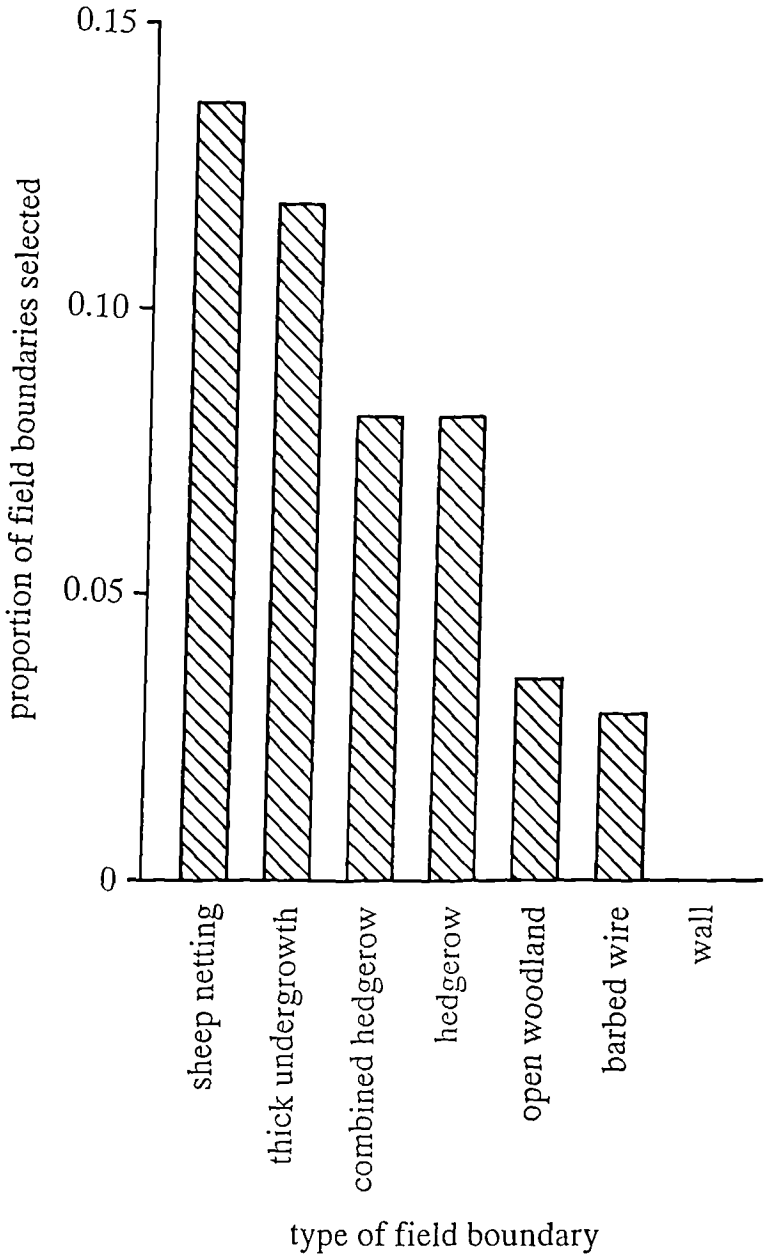
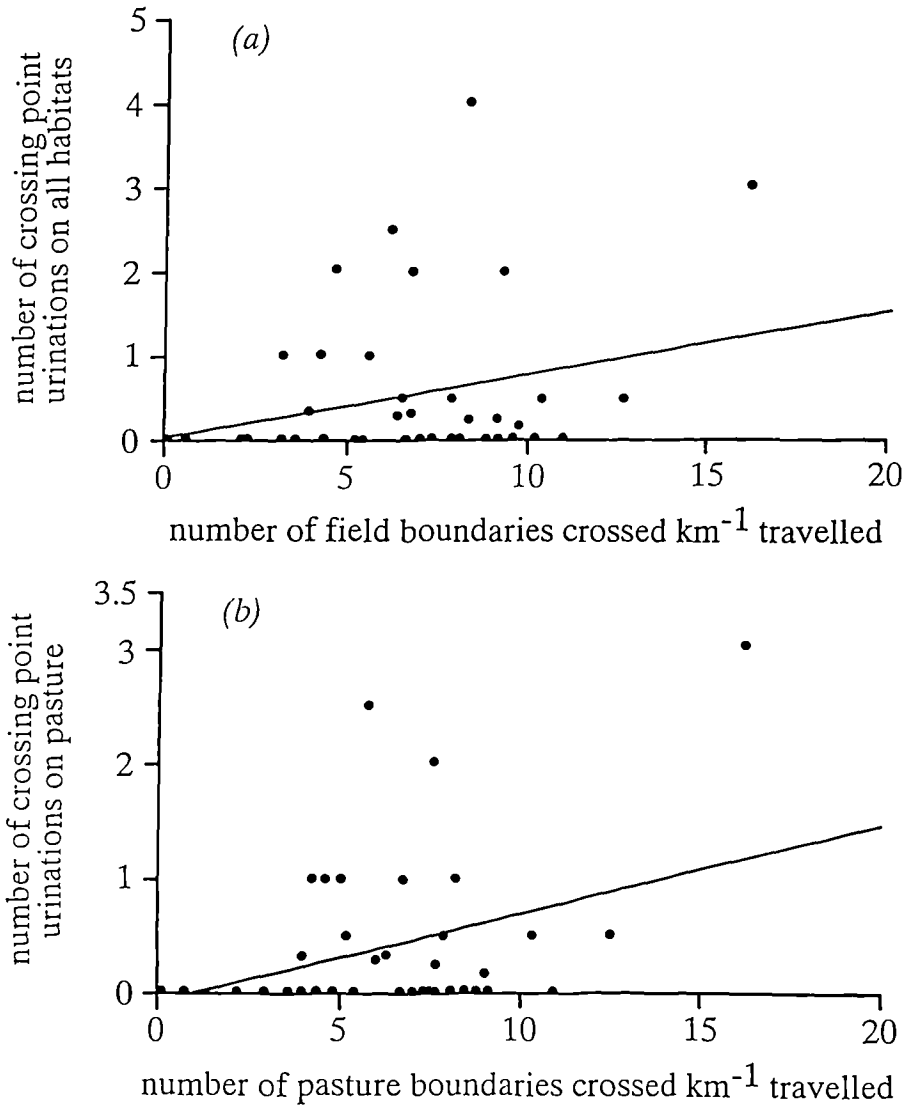


Figure 4.3 Relationship between the number of crossing point urinations and the number of field boundaries crossed km^{-1} travelled for (a) all habitat types ($F=4.71$, $r=0.33$, d.f. $=1,40$, $p<0.05$) and (b) pasture only ($F=7.46$, $r=0.40$, d.f. $=1,40$, $p<0.01$).



summer also received a significantly greater mean proportion of total defecations per night than in winter ($H=12.88$, d.f. $=3$, $p<0.01$).

4.3.4 Seasonal pattern of excretion on setts

Badgers were never observed to defecate on setts and only a relatively small number of urinations were located on all types of setts (Table 4.4). This behaviour was restricted to adult and yearling male badgers, with the mean number reaching a peak during the winter season. No significant seasonal variation was detected ($H=1.72$, d.f. $=3$, $p>0.05$).

4.3.5 Seasonal pattern of excretion on boundary runs

As with urination on setts, boundary run urinations occurred very infrequently throughout the year and this particular behaviour was restricted entirely to adult male badgers (Table 4.5). No seasonal trend in the mean number of boundary run urinations was detected ($H=2.60$, d.f. $=3$, $p>0.05$). Faeces located on the boundary were always deposited at latrines and never on the boundary path itself.

4.3.6 Seasonal pattern of excretion on pasture and woodland

No significant seasonal variation was detected in the mean number of urinations deposited per night in woodland ($H=4.81$, d.f. $=3$, $p>0.05$), although the mean proportion of urinations deposited in woodland reached a peak in the winter which was significantly greater than summer and autumn. The mean proportion of urine deposited in woodland in the spring was also significantly greater than in the summer ($H=8.95$, d.f. $=3$, $p<0.05$) (Table 4.6). In spring and

Table 4.4 Seasonal pattern of urination on setts (all types); figures are the mean number per badger per night ± s.e. (above) and the mean proportion of total urinations per badger per night ± s.e. (below).

Spring	Summer	Autumn	Winter
0.03 ± 0.03	0.06 ± 0.06	0.06 ± 0.06	0.10 ± 0.07
0.02 ± 0.02	0.13 ± 0.13	0.10 ± 0.11	0.09 ± 0.06

Table 4.5 Seasonal pattern of boundary run urinations (urine is at least 5m from the closest latrine); figures are the mean number of urinations per badger per night ± s.e. (above) and the mean proportion ± s.e. of total urinations per badger per night (below).

Spring	Summer	Autumn	Winter
0.05 ± 0.05	0.06 ± 0.06	0.06 ± 0.06	0.10 ± 0.10
0.02 ± 0.02	0.02 ± 0.02	0.10 ± 0.11	0.05 ± 0.05

Table 4.6 Seasonal pattern of urination on pasture and woodland; figures are the mean number of urinations per badger per night ± s.e. (above) and the mean proportion ± s.e. of total urinations per badger per night (below).

	Spring	Summer	Autumn	Winter
Pasture	1.10 ± 0.33	1.41 ± 0.53	0.96 ± 0.60	0.28 ± 0.16
	0.48 ± 0.10	0.83 ± 0.08	0.62 ± 0.15	0.33 ± 0.15
Woodland	0.86 ± 0.19	0.38 ± 0.21	0.24 ± 0.11	0.63 ± 0.20
	0.52 ± 0.10	0.17 ± 0.08	0.33 ± 0.16	0.63 ± 0.14

summer, badgers urinated significantly more times per night on pasture than during the winter season ($H=8.56$, d.f. $=3$, $p<0.05$), while the mean proportion of urine deposited on pasture in the summer was significantly greater than in the winter ($H=9.87$, d.f. $=3$, $p<0.05$).

The mean number of faeces deposited per night in woodland reached a peak during the winter/spring period (Table 4.7), although there was no significant seasonal variation. In contrast the mean proportion of faeces deposited in woodland was significantly higher in the winter than the autumn season ($H=11.26$, d.f. $=3$, $p<0.05$). Pasture defecations reached a peak in the autumn with the mean number per night during that season being significantly greater than in the winter ($H=9.02$, d.f. $=3$, $p<0.05$). A similar trend was observed in the mean proportion of defecations on pasture, although the proportion in summer was also significantly higher than winter ($H=12.88$, d.f. $=3$, $p<0.01$).

The number of defecations and urinations occurring on other habitat types such as arable land, leys and scrub were infrequent and showed no significant seasonal variation in either mean number deposited per night, nor in the mean proportion per night.

4.3.7 Seasonal pattern of urine trail and patch production

The mean number of urine trails and patches deposited per night on a badger's territory showed no significant seasonal variation (Table 4.8). The mean proportion of urinations in the form of trails rose gradually from spring onwards, reaching a peak in the autumn. A significantly higher proportion of urinations were deposited as trails during the autumn than in the winter season ($H=10.10$, d.f. $=3$, $p<0.05$). Conversely the proportion of patches produced in the winter was significantly higher than in the autumn.

Table 4.7 Seasonal pattern of defecation on pasture and woodland; figures are the mean number of defecations per badger per night ± s.e. (above) and the mean proportion ± s.e. of total defecations per badger per night (below).

	Spring	Summer	Autumn	Winter
Pasture	0.35 ± 0.11	0.35 ± 0.15	0.50 ± 0.13	0.05 ± 0.05
	0.37 ± 0.12	0.59 ± 0.19	0.73 ± 0.13	0.05 ± 0.05
Woodland	0.54 ± 0.12	0.15 ± 0.09	0.12 ± 0.08	0.50 ± 0.14
	0.63 ± 0.12	0.28 ± 0.17	0.15 ± 0.11	0.85 ± 0.11

Table 4.8 Seasonal pattern of excretion of urine trails and patches; figures are the mean number per badger per night ± s.e. (above) and the mean proportion of total urinations per badger per night ± s.e. (below).

	Spring	Summer	Autumn	Winter
Trail	1.33 ± 0.28	1.32 ± 0.54	1.00 ± 0.48	0.40 ± 0.19
	0.66 ± 0.08	0.67 ± 0.13	0.75 ± 0.14	0.26 ± 0.11
Patch	0.63 ± 0.15	0.53 ± 0.24	0.25 ± 0.14	0.60 ± 0.18
	0.34 ± 0.08	0.33 ± 0.13	0.25 ± 0.14	0.74 ± 0.11

4.3.8 Distribution of trails and patches within territories

The mean number of urine trails and urine patches deposited per badger were pooled seperately for latrines and runs within each season (Table 4.9). This accounted for some badgers being followed on more than one occasion within a season. Expected values were determined from the pooled mean number of trails and pooled mean number of patches produced within each season. In each season the number of trails and patches deposited at latrines showed no significant difference to that expected (spring, $X^2=0.01$; summer, $X^2=2.90$; autumn, $X^2=0.26$; winter, $X^2=1.09$; in all cases d.f.$=1$, $p>0.05$). Similarly for urinations on runs, no significant difference could be detected in any season between the observed number of trails and patches and that expected.

4.3.9 Seasonal pattern of habitat selection

Within each season the mean number of urinations on pasture and woodland were pooled separately and compared to the pooled mean distances travelled (km) in pasture and woodland (Table 4.10). No significant difference between observed and expected pooled means was detected in any season (spring, $X^2=0.14$; summer, $X^2=0.94$; autumn, $X^2=0.26$; winter, $X^2=1.09$; in all cases d.f.$=1$, $p>0.05$). This procedure was repeated for defecations on pasture and woodland (Table 4.11), and revealed that in the winter badgers defecated significantly more in woodland than expected on the basis of distance travelled in that particular habitat ($X^2=4.59$, d.f.$=1$, $p<0.05$).

Table 4.9 Seasonal distribution of urine trails and patches on runs and latrines (top values) and expected distribution (bottom values) based on total number of trails and patches produced within each season; figures are pooled mean number of urinations per badger per night. (Insufficient data were available for the analysis of distribution on runs in the autumn.)

		Spring	Summer	Autumn	Winter
Run					
	Trail	10.00	7.50	-	6.50
		10.61	9.45		5.29
	Patch	6.00	7.00	-	5.00
		5.39	5.05		6.21
Latrine					
	Trail	14.67	14.00	11.00	1.50
		14.47	11.77	11.70	2.83
	Patch	6.50	1.00	4.25	7.00
		6.70	3.23	3.55	5.67

Table 4.10 Expected pattern of defecation on pasture and woodland (bottom values), based on distances travelled within those habitats, in relation to that actually observed (top values); figures are pooled mean number of defecations per badger per night.

	Spring	Summer	Autumn	Winter
Pasture	7.00	5.95	8.50	1.00
	8.24	5.77	7.98	3.62
Woodland	10.80	2.55	2.04	10.00
	9.56	2.73	2.56	7.38

Table 4.11 Expected pattern of urination on pasture and woodland (bottom values), based on distances travelled within those habitats, in relation to that actually observed (top values); figures are pooled mean number of defecations per badger per night.

	Spring	Summer	Autumn	Winter
Pasture	22.00	23.97	16.32	5.60
	21.27	22.41	15.63	7.12
Woodland	17.20	6.46	4.08	12.60
	17.93	8.02	4.77	11.08

4.3.10 Stimuli releasing marking behaviour

One-way analysis of variance revealed no significant differences in any season in the various features, such as the number of fresh/old faeces and pits (Table 4.12) recorded at latrines (boundary and hinterland grouped) between latrines containing fluorescent urine from spool-and-line tracked animals and latrines previously visited but not selected for urinating. However, a different pattern emerged with respect to the badgers dunging behaviour (Table 4.13). In the spring season focal animals defecated at latrines containing significantly more fresh faeces than latrines visited earlier but not used for elimination of faeces (one-way ANOVA, $F=6.31$, d.f. $=1,20$, $p<0.05$). This was repeated in the summer (one-way ANOVA, $F=75.38$, d.f. $=1,10$, $p<0.0001$), when badgers also selected latrines containing significantly more faeces in total (one-way ANOVA, $F=11.18$, d.f. $=1,10$, $p<0.01$). The mean number of anal gland secretions (total) and the mean number attached to faeces were also significantly greater at latrines used to deposit faeces. During the autumn badgers defecated at latrines containing significantly more pits in total (one-way ANOVA, $F=6.28$, d.f. $=1,20$, $p<0.05$). During both the autumn and winter seasons, the total number of faeces and number of fresh faeces were significantly greater at latrines used for defecation.

When boundary and hinterland latrines were analysed separately, neither latrine type receiving urine showed any significant differences to those not selected for urinating by focal animals (Table 4.14). Badgers defecated at hinterland latrines containing a significantly greater mean number of faeces in total and mean number of fresh faeces (one-way ANOVA, $F=30.36$, d.f. $=1,44$, $p<0.0001$) (Table 4.15). Boundary latrine defecations occurred at latrines containing significantly more fresh faeces than those not used for eliminating faeces (one-way ANOVA, $F=10.45$, d.f. $=1,20$, $p<0.01$).

Table 4.12 Characteristics of latrines (boundary and hinterland grouped) selected for urinating (above) and those previously visited but not selected for urination (below); with the exception of scratching (mean score ± s.e.), figures are the mean number per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	5.44 ± 0.99	1.60 ± 0.97	2.40 ± 0.84	5.25 ± 0.87
	5.33 ± 0.85	2.40 ± 0.57	2.00 ± 0.79	3.25 ± 0.55
No. fresh pits	1.33 ± 0.81	0.80 ± 0.65	0.80 ± 0.42	2.00 ± 0.47
	1.00 ± 0.40	0.60 ± 0.27	0.80 ± 0.55	0.75 ± 0.87
No. old pits	4.11 ± 0.45	0.80 ± 0.42	1.60 ± 0.76	3.25 ± 0.73
	4.33 ± 0.87	1.80 ± 0.42	1.20 ± 0.42	2.50 ± 1.11
No. faeces (total)	5.44 ± 1.08	2.60 ± 0.97	9.60 ± 5.56	6.75 ± 2.18
	5.89 ± 0.86	2.60 ± 0.45	4.20 ± 1.39	3.00 ± 1.25
No. fresh faeces	1.22 ± 0.68	0.20 ± 0.22	2.20 ± 0.89	2.75 ± 1.66
	2.56 ± 0.71	1.00 ± 0.61	1.80 ± 0.55	1.00 ± 0.82
No. old faeces	4.22 ± 0.70	2.40 ± 0.91	7.40 ± 5.50	4.00 ± 0.82
	3.33 ± 0.66	1.60 ± 0.45	2.40 ± 0.91	2.00 ± 0.82
No. anal gland secretions (total)	0.78 ± 0.34	0.20 ± 0.22	0.40 ± 0.27	1.25 ± 1.44
	1.33 ± 0.61	0	0.20 ± 0.22	0.25 ± 0.29
No. anal gland secretions attached to faeces	0.56 ± 0.31	0.20 ± 0.22	0.20 ± 0.22	1.00 ± 1.15
	1.00 ± 0.61	0	0.20 ± 0.22	0.25 ± 0.29
No. anal gland secretions alone	0.22 ± 0.24	0	0.20 ± 0.22	0.25 ± 0.29
	0.33 ± 0.18	0	0	0
Scratching	0	0	0	0.50 ± 0.58
	0.11 ± 0.12	0	0	0

Table 4.13 Characteristics of latrines (boundary and hinterland grouped) selected for defecating (above) and those previously visited but not selected for faecal deposition (below); with the exception of scratching (mean score ± s.e.) and mean proportion of faeces in pits ± s.e., figures are the mean number per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	5.55 ± 0.77	5.67 ± 2.22	2.82 ± 0.58	4.75 ± 1.52
	5.73 ± 0.75	2.67 ± 1.01	1.27 ± 0.29	5.00 ± 2.45
No. fresh pits	1.09 ± 0.30	2.83 ± 1.31	1.18 ± 0.31	1.00 ± 0.82
	1.00 ± 0.37	0.83 ± 0.52	0.45 ± 0.17	2.25 ± 2.23
No. old pits	4.45 ± 0.59	2.83 ± 0.96	1.64 ± 0.35	3.75 ± 1.91
	4.73 ± 0.66	1.83 ± 0.96	0.82 ± 0.19	2.75 ± 0.55
No. faeces (total)	8.45 ± 1.32	7.50 ± 1.62	8.27 ± 1.41	7.75 ± 1.72
	6.36 ± 0.89	2.17 ± 0.66	4.64 ± 1.10	3.25 ± 0.87
No. fresh faeces	2.82 ± 0.69	5.33 ± 0.54	4.55 ± 0.71	4.25 ± 0.29
	1.00 ± 0.32	0.67 ± 0.23	1.00 ± 0.24	2.00 ± 0.82
No. old faeces	5.64 ± 0.94	2.17 ± 1.25	3.73 ± 1.01	3.50 ± 1.80
	5.36 ± 0.85	1.50 ± 0.73	3.64 ± 0.96	1.25 ± 0.55
No. anal gland secretions (total)	1.18 ± 0.40	1.00 ± 0.40	0.18 ± 0.13	1.50 ± 0.58
	0.73 ± 0.29	0	0.18 ± 0.13	0.25 ± 0.29
No. anal gland secretions attached to faeces	0.73 ± 0.40	0.67 ± 0.23	0.09 ± 0.10	1.25 ± 0.55
	0.18 ± 0.13	0	0.18 ± 0.13	0.25 ± 0.29
No. anal gland secretions alone	0.45 ± 0.22	0.33 ± 0.23	0.09 ± 0.10	0.25 ± 0.29
	0.55 ± 0.22	0	0	0
Proportion of faeces in pits	0.92 ± 0.04	0.58 ± 0.12	0.33 ± 0.07	0.89 ± 0.04
	0.99 ± 0.01	0.71 ± 0.18	0.23 ± 0.07	0.95 ± 0.06
Scratching	0	0	0	0.25 ± 0.29
	0	0	0	0

Table 4.14 Characteristics of boundary and hinterland latrines (grouped separately throughout year) selected for urinating (above) and not selected for urine deposition (below); with the exception of scratching (mean score ± s.e.), figures are the mean number per latrine ± s.e.

	Boundary latrine	Hinterland latrine
No. pits (total)	2.57 ± 0.78	4.56 ± 0.66
	6.14 ± 2.60	4.22 ± 0.60
No. fresh pits	0.29 ± 0.20	1.78 ± 0.45
	1.57 ± 0.78	1.34 ± 0.32
No. old pits	2.29 ± 0.81	2.78 ± 0.37
	4.57 ± 2.32	2.88 ± 0.55
No. faeces (total)	7.57 ± 4.00	4.81 ± 0.64
	5.00 ± 1.27	4.00 ± 0.58
No. fresh faeces	1.29 ± 0.73	1.63 ± 0.45
	2.71 ± 0.90	1.72 ± 0.40
No. old faeces	6.29 ± 3.80	3.19 ± 0.41
	2.29 ± 0.65	2.28 ± 0.37
No. anal gland secretions (total)	0.43 ± 0.32	0.53 ± 0.32
	0.57 ± 0.32	0.75 ± 0.36
No. anal gland secretions attached to faeces	0.43 ± 0.32	0.44 ± 0.27
	0.14 ± 0.15	0.53 ± 0.32
No. anal gland secretions alone	0	0.09 ± 0.07
	0.43 ± 0.22	0.50 ± 0.34
Scratching	0	0
	0	0

Table 4.15 Characteristics of boundary and hinterland latrines (grouped separately throughout the year) selected for defecation (above) and not selected for faecal deposition (below); with the exception of scratching (mean score ± s.e.), figures are the mean number per latrine ± s.e.

	Boundary latrine	Hinterland latrine
No. pits (total)	5.00 ± 1.20	4.74 ± 0.56
	3.14 ± 0.75	3.26 ± 0.52
No. fresh pits	2.00 ± 0.72	1.30 ± 0.24
	1.09 ± 0.43	0.87 ± 0.24
No. old pits	3.00 ± 0.65	3.44 ± 0.52
	2.05 ± 0.60	2.39 ± 0.48
No. faeces (total)	7.50 ± 1.17	7.57 ± 0.86
	4.82 ± 0.69	4.48 ± 0.60
No. fresh faeces	3.86 ± 0.83	3.65 ± 0.39
	1.18 ± 0.28	1.20 ± 0.24
No. old faeces	3.64 ± 0.68	3.91 ± 0.69
	3.64 ± 0.71	3.28 ± 0.60
No. anal gland secretions (total)	0.64 ± 0.25	0.57 ± 0.22
	0.27 ± 0.15	0.41 ± 0.15
No. anal gland secretions attached to faeces	0.36 ± 0.20	0.43 ± 0.20
	0.09 ± 0.10	0.24 ± 0.09
No. anal gland secretions alone	0.27 ± 0.15	0.13 ± 0.07
	0.18 ± 0.13	0.17 ± 0.10
Proportion of faeces in pits	0.73 ± 0.12	0.70 ± 0.07
	0.61 ± 0.12	0.66 ± 0.08
Scratching	0	0
	0	0

4.3.11 Number of latrine visits before deposition of faeces and urine

The mean number of latrines visited (boundary and hinterland) before defecation by the focal animal rose gradually from spring onwards, reaching a peak in the autumn, before dropping sharply in the winter (Table 4.16). There was, however, no significant seasonal variation (Kruskal-Wallis, $H=4.17$, $d.f.=3$, $p>0.05$). Throughout the year, badgers visited significantly more hinterland latrines (1.47 ± 0.23) before defecating than boundary latrines (0.69 ± 0.35) ($H=5.41$, $d.f.=1$, $p<0.05$). Badgers also during the year visited more hinterland latrines (0.90 ± 0.24) than boundary latrines (0.44 ± 0.26) before urinating, however this difference was not significant ($H=1.01$, $d.f.=1$, $p>0.05$).

4.3.12 *Seasonal pattern of behaviour at latrines*

In each season a large proportion of faeces were deposited either on top of or beside fresh faeces already present at latrines, with the peak occurring in the summer (Table 4.17). During this season all marked faeces were excreted on fresh faeces, although no significant trend was detected (Kruskal-Wallis, $H=7.74$, $d.f.=3$, $p>0.05$), nor when the summer and autumn seasons were combined and compared to the combined winter/spring period. The mean proportion of marked faeces deposited in pits at latrines reached a peak in the spring when all faeces produced by focal animals were deposited in pits. No significant seasonal variation was detected ($H=4.56$, $d.f.=3$, $p>0.05$). Those faeces from focal badgers not found in pits were deposited directly onto the ground surface at the latrine.

Table 4.16 Seasonal variation in the number of latrines (boundary and hinterland grouped) visited before focal animals defecated (above) and urinated (below); figures are the mean number per badger per night ± s.e.

Spring	Summer	Autumn	Winter
1.24 ± 0.28	1.63 ± 0.49	1.82 ± 0.69	0.75 ± 0.38
1.73 ± 0.43	1.00 ± 0.61	0.40 ± 0.27	1.17 ± 0.52

Table 4.17 Seasonal pattern of behaviour at latrines; (a) seasonal variation in the number of faeces produced by focal animals deposited in pits; (b) number of urinations deposited in pits; (c) number of faeces deposited next to/on top of fresh faeces already present at latrines; figures are the mean number per badger per night ± s.e. (above) and mean proportions ± s.e. (below).

	Spring	Summer	Autumn	Winter
(a)	0.97 ± 0.14	0.38 ± 0.15	0.50 ± 0.15	0.60 ± 0.14
	1.00 ± 0	0.79 ± 0.16	0.67 ± 0.15	0.97 ± 0.03
(b)	0.03 ± 0.03	0.06 ± 0.06	0	0.18 ± 0.09
	0.03 ± 0.03	0.06 ± 0.07	-	0.32 ± 0.15
(c)	0.80 ± 0.14	0.47 ± 0.16	0.65 ± 0.15	0.40 ± 0.12
	0.84 ± 0.09	1.00 ± 0	0.88 ± 0.10	0.70 ± 0.14

4.4 Discussion

Due to the difficulty in locating urine in the field, there is little quantified data on the pattern of urine distribution on a badgers territory, yet urine appears to be more important than faeces in the transmission of bovine tuberculosis from badgers to cattle (Brown, Harris & Cheeseman 1993). The importance of urine as a scent marker in badgers, particularly in the territorial behaviour of adult males has only recently been established (Brown, Cheeseman & Harris 1993), and the production of urine by different age and sex categories of badger was considerably more varied than for faeces, both between age and sex categories and between seasons.

The distribution of urine on a badgers territory was also varied, relative to faeces, with its deposition occuring at a much wider range of sites. During the spring, summer and winter seasons urine was deposited at latrines and on runs significantly more times per night than random urinations. However, during the autumn there was no significant difference detected between the mean number of urinations per night between these three sites. This was due to the behaviour of cubs in this season, which deposited urine entirely at random on pasture. The behaviour of badger cubs in the autumn is potentially of major significance in the transmission of bovine tuberculosis to cattle, due to the random distribution of urine on pasture and also given the high incidence of bovine tuberculosis found in cubs in parts of the south-west. Cheeseman *et al.* (1981) found 36% of cubs to be infected, compared with 19% of adults, indicating that *M. bovis* may be excreted onto pasture in a random fashion which may increase the probability of contact by cattle. Random urinations probably pose a greater risk to cattle since they do not occur repeatedly at a strategic site such as a latrine where the higher levels of contamination with both urine and faeces will increase their conspicuousness to

cattle. However, although random urinations are potentially important they occured infrequently and during a period when many cattle were being taken off pasture.

Faeces were deposited almost exclusively at latrines, with only a single defecation occuring on a crossing point run during the winter. Concentrating faeces at certain heavily reused sites has also been reported by Peters & Mech (1975) in wolves and in coyotes by Camenzind (1978). This behaviour seems to have reached its highest level in the spotted hyaena (Kruuk 1972). In badgers, the mean number of urinations per night at latrines on pasture reached a peak in the summer, with over 40% of total urinations per night occurring at pasture latrines during this season, declining to a very low level in the winter. It is difficult to assess the potential risk of urine at latrines to cattle. As latrines are generally regularly used sites and visually conspicuous, cattle may learn of there location on pasture and thus avoid them more readily than excreta on pasture away from latrines. The presence of faeces at latrines, which are more strongly avoided than urine (Benham & Broom 1991), may result in cattle not coming into contact with a relatively large proportion of the urine located at latrines. Benham & Broom (1991) found that although many individuals obviously detected the presence of badger excreta at latrines and strongly avoided it, a small number of individuals apparently grazed through the latrine area without concern. Even if the majority of cattle avoid grazing at latrines, during the sniffing process infected material may be inhaled by cattle, which are very susceptible to infection by the respiratory tract (Francis 1971). Denying cattle access to badger latrines would considerably reduce their exposure to badger excreta particularly during summer and autumn. It is, however, important that badgers at the same time are not excluded from latrines, since this may result in a more unpredictable situation, with excreta deposition occurring elsewhere on pasture.

Although not significant, the mean number and mean proportion of urinations on crossing point runs on all habitats was at its highest in the summer.

Urinating at such sites would maximise the chance of the scent mark being detected by conspecifics as badgers travelling across field boundaries converge at these sites to move from field to field, field to woodland and vice-versa. Similarly red foxes (Macdonald 1980) and wolves (Peters & Mech 1975) often travel along well-used trails and leave their scent marks on these routes and at junctions between them. Pine martens occasionally use the ski trails of trackers, but regularly scent-mark the junctions between these and their own trails. These scent marks are believed to play an important role in the pine martens spatial organization, so that it 'reads' other individuals tracks and learns whether it is worth hunting in the area or not (Pulliainen 1982).

The badger urine mark on crossing point runs may transmit information on the length of time that has elapsed since a badger passed that way. As the number of crossing point urinations reached a maximum in the summer during a period when food availability is low, providing information on how recently other animals passed, where others are foraging, or have foraged in the recent past, will enable an animal to avoid areas which are likely to be unproductive and hence increase their foraging efficiency. Alternatively, rather than an altruistic behaviour, badgers may deposit urine on crossing point runs during the summer when food availability is at its lowest in order to defend food patches from other members of the group or from neighbouring animals.

The peak in urinations on pasture crossing points occurred in the spring and although an overall significance was detected, this season was not significantly distinct from any other. The relevance of this peak at this time of year is borne out when considering the time of year that cattle are at greatest risk to infection. Wilesmith *et al.* (1982) have pointed out that cattle are at greatest risk in April and May, and suggest that there was re-exposure to infection at this time each year. The cattle were apparently exposed to *M. bovis* at sufficiently high levels for transmission to occur over a relatively short period of time. They suggested that

cattle were re-exposed to infection at the beginning of the grazing season each year and pointed out that re-exposure to infection in each year was not entirely coincident with a re-exposure to pasture as young stock that were out-wintered were also infected during this period.

Urine left on pasture seems to be the most likely candidate in the transmission process and, during this study, cattle were observed to eat pasture contaminated with urine at crossing point runs within 24 hours of deposition. Linear features which restrict badger movements to a limited number of crossing points (sheep netting, hedges, thick undergrowth) are particularly important, although the type of critical linear feature is likely to vary regionally. Walls were not considered to restrict badger movements because at the study site large sections of dry stone walls had collapsed. Gates were also common in these walls, which were used by badgers on 40% of crossings.

The correlation between the number of crossing point urinations and the number of linear features crossed suggests that a similar relationship may occur in other problem areas of the south-west. If so, this is probably the key factor in determining whether these crossing point urinations really are important in the transmission process. White, Brown & Harris (1993) have shown that areas in which cattle are at high risk from infection do not have higher badger population densities, nor a higher proportion of habitats favourable to badgers, but do have a greater degree of habitat heterogeneity and more linear features. The hypothesis is therefore presented that these crossing point urinations pose a significant risk to cattle, and that areas with increased numbers of linear features have greater levels of contamination of pasture with badger urine and hence a greater chance of disease transmission.

Obviously, other factors will also play a role in the transmission process. Although at present there is no evidence of a relationship between badger group size and the prevalence of bovine tuberculosis in badgers (Cheeseman *et al.* 1988), it is

still possible that there is an enhanced disease risk with larger badger social groups and that there is some relationship between group size and habitat (Kruuk 1978; Kruuk & Parish 1982; Hofer 1988), although data on any such relationships are scant. In fact Cheeseman *et al.* (1987) have shown that badger social group size exhibits large variations from year to year, and can undergo significant long-term changes in the absence of any habitat changes.

Although over 90% of crossing point urinations on pasture would be excluded from cattle if cattle were denied axcess to an area 4 m from a field boundary, a small proportion of urinations began up to 7.5 m into the field. The major reason that such a large area would need to be excluded from cattle was that badgers frequently produced trails of urine that extended into the field. Although 35.7% of all urinations on crossing point runs began within 1 m of a field boundary, only 17.9% of all urinations at crossing points would be totally excluded from cattle at that distance. A feature of urine trails that may be of significance in the transmission process was that small droplets of urine commonly extended for several metres at the end of trails. Even though cattle generally avoided badger urine (Benham & Broom 1991), these droplets may be small enough to go unnoticed. Although comparatively high numbers of cattle in certain limited areas in the south-west continue to fail the tuberculin test, the risk of infection in these areas is still low. The relatively low number of urinations on pasture would account for the low risk of transmission even in the problem areas, since the chance of cattle eating contaminated pasture whilst the bacilli are still infective is low, especially if a high proportion of cattle avoid eating pasture contaminated with badger urine (Benham & Broom 1991).

As well as the peak rise of infection and the re-exposure of cattle coinciding with the peak in crossing point urinations on pasture, they are also coincident with the peak in potential exposure of *M. bovis* from badgers. In Gloucestershire and Avon the peak prevalence of badgers with advanced tuberculous pneumonia was

found to occur in badgers caught or found dead in April, with 43% of all tuberculous animals caught in March and April (J Gallagher unpublished).

The length of time any excreted *M. bovis* survive on pasture is an important factor in the transmission of infection from badgers to cattle. The organism in badger urine and sputum on pasture remains viable for only a few days in summer and up to 10 weeks in winter (Ministry of Agriculture, Fisheries and Food 1979). Although a peak in the mean number of urinations per night on pasture was observed during the summer and the mean proportion of urinations on pasture during this season was significantly greater than all other seasons, it is possible that infected urine deposited on pasture during the summer months posses relatively little risk to cattle due to the relatively poor survival of *M. bovis* during this period.

The low level of pasture contamination during the winter is consistent with the observations of Wilesmith *et al.* (1982), who report that cattle are at little or no risk of becoming infected during the winter months, the risk declining from a peak in late spring throughout the remainder of the summer and following winter. In the present study the majority of excreta produced during the winter was deposited in woodland. In fact during this period badgers defecated significantly more in woodland than expected on the basis of distance travelled in this particular habitat. The pattern of defecation at this time of year is probably explained by the reduced activity seen in badgers during the winter (Harris 1982), with animals generally defecating at the first latrine visited, usually situated within woodland.

In each season badgers defecated at latrines (boundary and hinterland grouped) that contained significantly more fresh faeces than those latrines previously visited but not selected for faecal deposition. No significant differences however, were detected between the contents of latrines receiving urine and those not used for urinating. Faeces were therefore not responsible for eliciting urination. This was probably produced in response to urine already located at latrines which could not be recorded. Yamamoto & Hidaka (1984) found that raccoon dogs

preferred to deposit their faeces on a site where dung piles already exist. They suggested that the latrine is utilized as a place of information exchange about conspecific individuals, as raccoon dogs are able to distinguish the faeces of unknown conspecifics from their own at the latrine. By moving latrines utilised by captive raccoon dogs, Yamamoto and Hidaka (1984) revealed that the presence of a dung pile and not the location of the latrine was important for the animals in their choice of elimination site. Further work is needed to investigate the effects on badger excretory behaviour of manipulating badger latrines by the addition or removal of faeces and urine, as well as monitoring the effects of moving or removing entire latrines. It may be possible using such techniques to reduce the level of contamination of pasture by badger excreta, and increase excretory behaviour in areas not accessible to cattle, such as woodland. Achieving this should help reduce the incidence of TB in cattle.

In conclusion, since White, Brown & Harris (1993) have shown that habitat features are correlated with herds with reactor cattle, the urinatory behaviour of badgers on crossing point runs, as determined by the type and density of linear habitat features, is probably the significant causative factor behind this correlation. However, more detailed information is needed on the nature of boundary differences between different regions of the south-west and on the pattern of crossing point urinations in different habitat types. Improving the understanding of the relationship between habitat and the risk of transmission will provide a basis on which to develop a more effective method of reducing the number of reactor cattle in the south-west.

CHAPTER 5

LATRINE ACTIVITY AND ITS ROLE IN TERRITORIALITY AND TB TRANSMISSION

5.1 Introduction

Badgers defecate at small open pits and at particular sites within a group's territory these pits are aggregated to form latrines. Latrines may vary considerably in size, in both area, and in number of pits, with some latrines reported to contain up to 50 separate pits within a few square metres (Neal 1977; Kruuk 1978).

Apart from defecations badgers may also deposit secretions from their anal glands and from subcaudal glands at latrines (Neal 1977; Kruuk 1978; Kruuk, Gorman & Leitch 1984). Anal gland secretions are frequently deposited on top of faeces (Neal 1977; Roper, Shepherdson & Davies 1986), but can also be deposited alone in separate pits (Kruuk 1978; Roper, Shepherdson & Davies 1986). Badgers may scratch at the surface of the ground in the vicinity of the latrine, a behaviour probably associated with the release of secretions from the inter-digital glands. Badgers also urinate at latrines, either on the surrounding ground, or in specialised pits (i.e. pits not used for defecation and/or anal gland secretion) (Paget & Middleton 1974). Thus an active latrine forms both a visual and olfactory landmark.

For badgers living at high densities in rural areas, latrines are generally more frequent towards the edges of badger territories than in the centre (Kruuk 1978; Macdonald 1980). In such a situation, Kruuk (1978) found that well-defended territorial boundaries were demarcated by large, active latrine sites, and that latrines were smaller towards the centre of the range and near the setts. In

contrast to these strongly defended group territories, a latrine survey for a Bristol population of badgers revealed no obvious territorial boundaries even where social groups were contiguous. In this population ranges showed considerable overlap and latrines were accumulated around setts rather than along territorial boundaries (Cresswell & Harris 1988).

Boundary latrines may be visited by badgers from more than one social group (Kruuk 1978; Cheeseman *et al.* 1981), enabling inter-group transfer of olfactory information, and several studies have shown that latrine use reflects territoriality (Kruuk 1978; Cheeseman *et al.* 1981; Roper, Shepherdson & Davies 1986). Whilst there is a general agreement over the significance of latrines in territoriality, it is less clear which secretions and excretory products are important components of latrines in maintaining territories, nor is it clear how the use of latrines at certain times of the year reflects the degree of territorial behaviour at that time.

It is presumed that the majority of the cases of infection in cattle are due to the consumption of grass contaminated by badger urine, faeces or sputum (Muirhead, Gallagher & Burn 1974; Ministry of Agriculture, Fisheries and Food 1979), although the role of latrines on pasture in this process is largely unknown. Contact between cattle and latrines has been shown to depend on the location of the latrine within the field, and the age of the cattle (Benham & Broom 1991). Immediately after turn-out, younger cattle spent more time at latrines around the perimeter of fields, while older cattle spent more time investigating more central latrines. Cattle reacted to latrine areas with increased sniffing and reduced grazing compared to non-latrine areas, although a small proportion of cattle grazed latrine areas.

As described in the previous chapter, urine is probably the most significant badger excretory product involved in the transmission of bovine tuberculosis from badgers to cattle. In experimental studies, cattle strongly avoided the ingestion of

badger faeces (Benham & Broom 1991), with 99.3% of cows avoiding contaminated grass. In a similar experimental study, urine exposed to cattle was avoided by a lower proportion of cows (88.7%). The lower level of sniffing performed by cattle in response to faeces (Benham & Broom 1991) and its greater avoidance suggests that faeces are more easily detected than urine. Due to the strong avoidance of faeces, excretory products deposited by badgers at latrines, in particular infective urine, may be of less significance in the transmission process, especially at highly active latrines heavily contaminated with faeces.

Despite the possible lower risk of infection to cattle from latrines, compared to waste products deposited on pasture away from latrines, the presence of infective material at latrines still constitutes some risk to cattle, especially to cows that graze latrine areas. This chapter looks at the seasonal pattern of latrine use and its significance in territoriality, how the distribution of latrines is affected by particular features of the habitat and how the use of latrines might afford an increased risk of transmission.

5.2 Methods

For each focal badger that was spool-and-line tracked, the number of latrines visited, the number of defecations, urinations and anal gland secretions were measured nightly. The locations of all visited latrines and nightly movements were recorded on a map of scale 1:2500. Following a nights tracking, data were collected from all latrines visited by a focal badger. This therefore included latrines which had not been used by focal animals to deposit marked excreta or anal gland secretion. The data collected from latrines included: number of fresh faeces (judged to be less than three days old), number of old faeces, number of fresh pits (as for faeces), number of old pits, number of faeces in and out of pits, number of anal gland secretions associated with faeces and number alone and a score (0-5) of the

extent of scratching. Length of linear features and area of the major habitat types were calculated using a bit pad.

5.3 Results

Between June 1991 and May 1992, data were collected from 121 different latrine sites (total number of observations=243). Within each season the percentage of latrines visited were as follows: spring 77.3%, summer 79.3%, autumn 86.2% and winter 73.0%.

5.3.1 Seasonal pattern of latrine activity (all latrine categories)

Two-way analysis of variance revealed an overall significant difference between seasons in the number of fresh faeces per latrine ($F=2.95$, d.f.$=3,64$, $p=0.04$, Tukey's HSD), although due to the conservative nature of the Tukey test, no season could be isolated as being significantly distinct. The number of old faeces per latrine showed considerably more seasonal variation and reached a peak in spring and autumn (Table 5.1), with the number per latrine during these seasons being significantly greater than in the summer and winter seasons (two-way ANOVA, $F=5.52$, d.f.$=3,64$, $p=0.002$, Tukey's HSD). Although the total number of faeces per latrine reached a peak in the autumn, no significant seasonal variation was detected (two-way ANOVA, $F=1.94$, d.f.$=3,64$, $p>0.05$). The proportion of fresh faeces per latrine was at its lowest during spring and rose to approximately 40% for the remainder of the year, with the spring proportion significantly lower than that for the winter season (two-way ANOVA, $F=3.67$, d.f.$=3,59$, $p<0.05$, Tukey's HSD). Chi-square analysis for proportions revealed that although the percentage of latrines containing fresh faeces was lowest in the

Table 5.1 Seasonal pattern of latrine use (boundary and hinterland latrines grouped from all habitat types); with the exception of scratching (mean score per latrine ± s.e.) and the mean proportion of fresh faeces and faeces in pits per latrine ± s.e., figures are the mean no. per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	6.55 ± 0.45	2.44 ± 0.46	0.94 ± 0.21	5.38 ± 0.46
No. fresh pits	1.55 ± 0.19	0.67 ± 0.25	0.25 ± 0.10	2.18 ± 0.27
No. old pits	4.99 ± 0.37	1.76 ± 0.29	0.69 ± 0.16	3.20 ± 0.31
No. faeces (total)	5.66 ± 0.44	3.07 ± 0.45	6.28 ± 1.12	4.40 ± 0.57
No. fresh faeces	1.32 ± 0.17	1.27 ± 0.28	1.92 ± 0.31	1.96 ± 0.28
No. old faeces	4.34 ± 0.37	1.80 ± 0.30	4.36 ± 0.96	2.43 ± 0.34
Proportion of fresh faeces	0.24 ± 0.03	0.40 ± 0.07	0.40 ± 0.04	0.43 ± 0.05
No. anal gland secretions (total)	0.55 ± 0.11	0.34 ± 0.11	0.17 ± 0.06	0.57 ± 0.13
No. anal gland secretions attached to faeces	0.26 ± 0.09	0.18 ± 0.06	0.14 ± 0.06	0.37 ± 0.12
No. anal gland secretions alone	0.29 ± 0.06	0.16 ± 0.06	0.03 ± 0.03	0.20 ± 0.06
Proportion of faeces in pits	0.94 ± 0.02	0.59 ± 0.07	0.31 ± 0.05	0.91 ± 0.03
Scratching	0.07 ± 0.04	0	0	0.20 ± 0.06

summer (Table 5.2), no significant difference between seasons was detected (X^2=3.77, d.f.=3, p>0.05).

A significant seasonal variation was detected in the total number of pits per latrine (two-way ANOVA, F=14.43, d.f.=3,64, p=0.0001, Tukey's HSD). This analysis revealed that the number of pits per latrine during the spring was significantly greater than during any other season. The number of pits per latrine in the winter was also significantly greater than summer and autumn, with the number in summer significantly greater than the autumn. This seasonal variation was due largely to a variation in the number of old pits per latrine (two-way ANOVA, F=20.84, d.f.=3,64, p=0.0001, Tukey's HSD), with seasonal differences the same as for the previous analysis. Although fresh digging at latrines reached a peak during the winter/spring period, no significant seasonal variation was detected (two-way ANOVA, F=2.27, d.f.=3,64, p>0.05).

Also during the winter and spring seasons, badgers deposited a greater proportion of their faeces in pits than during the summer and autumn months when badgers frequently defecated away from pits at latrines, leaving their excreta on the surface of the ground (Kruskal-Wallis, H=53.66, d.f.=3, p<0.0001). This behaviour was particularly common during the autumn season.

No seasonal trend was detected in the number of anal gland secretions associated with faeces at latrines, although the number of anal gland secretions deposited separate to faeces was significantly higher in the spring than during the autumn (Kruskal-Wallis, H=11.02, d.f.=3, p<0.05). These anal gland secretions were frequently deposited alone in pits. Considering the two cases together, the overall output of anal gland secretion reached a peak during the winter season, although no significant seasonal variation was detected. Combining the winter and spring periods, anal gland secretion was significantly greater than in the combined summer and autumn periods (Kruskal-Wallis, H=6.57, d.f.=1, p<0.05). No

Table 5.2 Seasonal variation in the proportion of boundary and hinterland latrines containing (a) fresh faeces and (b) anal gland secretion throughout the year.

(a)

	Spring	Summer	Autumn	Winter
Boundary latrines	69.57	71.43	81.25	69.23
Hinterland latrines	68.57	58.82	83.33	68.18
Grouped	68.97	64.52	82.35	68.42

(b)

	Spring	Summer	Autumn	Winter
Boundary latrines	34.78	42.86	43.75	46.15
Hinterland latrines	45.71	17.65	27.78	34.09
Grouped	41.38	29.03	35.29	36.84

significant trend was detected in the percentage of latrines containing anal gland secretion (X^2=3.49, d.f.=3, p>0.05) (Table 5.2).

The scratching behaviour of badgers at latrines was only observed during the winter and spring seasons, being most extensive in the winter. The mean scratching score per latrine during this season was significantly greater than in the summer and autumn seasons (Kruskal-Wallis, H=11.63, d.f.=3, p<0.01).

5.3.2 *Seasonal pattern of boundary latrine activity*

The number of fresh faeces per latrine rose gradually from spring onwards, reaching a peak in the autumn, before dropping away in the winter (Table 5.3). Although a trend (p=0.08) was detected this was not significant (two-way ANOVA, F=2.57, d.f.=3,20, p>0.05). The percentage of boundary latrines containing fresh faeces also reached a peak during the autumn, although not significant (X^2=0.45, d.f.=3, p>0.05) (Table 5.2). No significant differences were detected in the number of old faeces per latrine, nor in the total number of faeces per latrine.

Again the digging of pits was more common during the winter and spring periods, although no significance was detected between seasons in the number of fresh pits per latrine (two-way ANOVA, F=2.22, d.f.=3,20, p>0.05). The number of old pits and total number of pits per latrine were significantly greater in spring and winter than summer and autumn (two-way ANOVA, F=5.16, d.f.=3,20, P<0.01, Tukey's HSD). The proportion of faeces in pits was significantly greater in spring and winter than autumn (Kruskal-Wallis, H=21.78, d.f.=3, p<0.0001).

The total number of anal gland secretions per boundary latrine varied little throughout the year (Kruskal-Wallis, H=3.25, d.f.=3, p>0.05), as did the percentage containing anal gland secretion (X^2=1.37, d.f.=3, p>0.05) (Table

Table 5.3 Seasonal pattern of boundary latrine use (all habitat types); with the exception of scratching (mean score per latrine ± s.e.) and the mean proportion of fresh faeces and faeces in pits per latrine ± s.e., figures are the mean no. per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	7.14 ± 0.91	2.61 ± 0.93	1.06 ± 0.35	6.77 ± 1.10
No. fresh pits	1.77 ± 0.33	0.82 ± 0.52	0.16 ± 0.13	2.85 ± 0.59
No. old pits	5.37 ± 0.79	1.79 ± 0.50	0.91 ± 0.29	3.92 ± 0.64
No. faeces (total)	5.35 ± 0.66	4.07 ± 0.84	7.66 ± 1.84	3.81 ± 1.12
No. fresh faeces	1.40 ± 0.27	1.79 ± 0.54	2.19 ± 0.53	1.50 ± 0.49
No. old faeces	3.96 ± 0.58	2.29 ± 0.52	5.47 ± 1.66	2.31 ± 0.72
Proportion of fresh faeces	0.26 ± 0.05	0.43 ± 0.10	0.34 ± 0.06	0.48 ± 0.11
No. anal gland secretions (total)	0.51 ± 0.18	0.50 ± 0.19	0.28 ± 0.12	0.65 ± 0.23
Proportion of faeces in pits	0.90 ± 0.05	0.55 ± 0.11	0.23 ± 0.06	0.98 ± 0.01
Scratching	0.15 ± 0.10	0	0	0.31 ± 0.13

5.2). However, scratching at boundary latrines was significantly greater during winter than in summer and autumn (Kruskal-Wallis, $H=11.27$, d.f. $=3$, $p<0.05$).

5.3.3 Seasonal pattern of hinterland latrine activity

Unlike the seasonal variation in fresh faeces at boundary latrines, the number of fresh faeces per hinterland latrine was at its lowest in the summer, rising to a peak in the winter, being significantly greater at this time than in spring and summer (two-way ANOVA, $F=4.49$, d.f. $=3,40$, $p<0.01$, Tukey's HSD) (Table 5.4).

The number of old faeces at hinterland latrines was also at its lowest level in the summer, with the number in this season significantly lower than in spring and autumn (two-way ANOVA, $F=4.11$, d.f. $=3,40$, $p<0.05$, Tukey's HSD). Despite significant trends in the number of fresh/old faeces, no significant variation was detected in the total number of faeces per latrine.

The number of fresh pits was significantly greater in the winter season than in summer and autumn (two-way ANOVA, $F=2.95$, d.f. $=3,40$, $p<0.05$, Tukey's HSD). The number of old pits and the total number of pits showed the same significant seasonal trends as for boundary latrines (see Tables 5.3 and 5.4).

Despite an overall significance in the seasonal proportion of fresh faeces at latrines (two-way ANOVA, $F=3.69$, d.f. $=3,36$, $p<0.05$, Tukey's HSD), no season could be isolated as being significantly distinct from any other. The proportion of faeces in pits was significantly higher in the spring than both summer and autumn, and the winter proportion significantly greater than in the autumn season ($H=32.44$, d.f. $=3$, $p<0.0001$). During spring significantly more anal gland secretions were recorded at hinterland latrines than during autumn, while no significant variation was apparent in the mean scratching score between seasons.

Table 5.4 Seasonal pattern of hinterland latrine use (all habitat types); with the exception of scratching (mean score per latrine ± s.e.) and the mean proportion of fresh faeces and faeces in pits per latrine ± s.e., figures are the mean no. per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	6.16 ± 0.46	2.29 ± 0.42	0.82 ± 0.26	4.97 ± 0.50
No. fresh pits	1.40 ± 0.23	0.55 ± 0.21	0.33 ± 0.14	1.98 ± 0.30
No. old pits	4.73 ± 0.35	1.75 ± 0.37	0.49 ± 0.17	2.99 ± 0.35
No. faeces (total)	5.86 ± 0.60	2.25 ± 0.39	5.06 ± 1.38	4.57 ± 0.67
No. fresh faeces	1.27 ± 0.23	0.85 ± 0.25	1.68 ± 0.35	2.10 ± 0.34
No. old faeces	4.59 ± 0.49	1.39 ± 0.33	3.38 ± 1.09	2.47 ± 0.39
Proportion of fresh faeces	0.23 ± 0.04	0.38 ± 0.11	0.44 ± 0.06	0.41 ± 0.05
No. anal gland secretions (total)	0.58 ± 0.15	0.21 ± 0.12	0.07 ± 0.06	0.55 ± 0.15
Proportion of faeces in pits	0.97 ± 0.01	0.62 ± 0.10	0.35 ± 0.08	0.90 ± 0.04
Scratching	0.01 ± 0.01	0	0	0.17 ± 0.07

5.3.4 Seasonal pattern of activity at latrines on pasture

Many of the significant trends detected in the analyses above were not apparent when examining pasture latrine activity. The number of fresh faeces increased from spring to a peak in the autumn, before falling sharply in the winter (Table 5.5). This trend, however, was not quite significant (two-way ANOVA, $F=2.97$, d.f. $=3,20$, $p=0.06$). As with previous analyses, the number of pits at latrines on pasture during winter and spring were significantly greater than in summer and autumn (two-way ANOVA, $F=3.72$, d.f. $=3,20$, $p<0.05$, Tukey's HSD). The digging activity of badgers (number of fresh pits) was at a very low level during the autumn period, although not significant. Also during the autumn, badgers infrequently deposited faeces in pits, resulting in significantly larger numbers of faeces occurring at latrines that were not located in pits, than during any other season (two-way ANOVA, $F=3.81$, d.f. $=3,20$, $p<0.05$, Tukey's HSD). These faeces, although situated at the latrine site, were deposited directly on to the surface of the ground and not deposited in pits.

5.3.5 Seasonal pattern of activity at latrines in woodland

Significantly larger numbers of old faeces were located at woodland latrines during spring than during any other season (two-way ANOVA, $F=4.46$, d.f. $=3,36$, $p<0.01$, Tukey's HSD), while no significant trend was detected in the number of fresh faeces (Table 5.6). Despite overall significance in the proportion of fresh faeces at latrines (two-way ANOVA, $F=2.95$, d.f. $=3,33$, $p<0.05$, Tukey's HSD), no season was significantly distinct. Although the number of faeces not deposited in pits reached a peak during the autumn, this behaviour was considerably less pronounced compared to pasture latrines during the same period, and no significant seasonal variation was detected (two-way ANOVA, $F=1.86$,

Table 5.5 Seasonal pattern of pasture latrine use; with the exception of scratching (mean score per latrine ± s.e.) and mean proportion of faeces in pits per latrine ± s.e., figures are the mean no. per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	5.35 ± 0.63	2.50 ± 0.72	0.70 ± 0.25	5.63 ± 0.93
No. fresh pits	1.07 ± 0.36	0.74 ± 0.39	0.08 ± 0.06	2.94 ± 0.76
No. old pits	4.29 ± 0.62	1.76 ± 0.43	0.63 ± 0.24	2.69 ± 0.66
No. faeces (total)	6.62 ± 0.82	3.63 ± 0.66	8.85 ± 1.64	2.69 ± 0.85
No. fresh faeces	1.25 ± 0.29	1.66 ± 0.43	2.38 ± 0.45	0.88 ± 0.37
No. old faeces	5.37 ± 0.78	1.97 ± 0.42	6.48 ± 1.45	1.81 ± 0.67
Proportion of faeces in pits	0.84 ± 0.07	0.59 ± 0.09	0.18 ± 0.04	0.82 ± 0.13
No. anal gland secretions (total)	0.45 ± 0.24	0.50 ± 0.16	0.18 ± 0.09	0.50 ± 0.29
Scratching	0	0	0	0.13 ± 0.13

Table 5.6 Seasonal pattern of woodland latrine use; with the exception of scratching (mean score per latrine ± s.e.) and the mean proportion of faeces in pits per latrine ± s.e., figures are the mean no. per latrine ± s.e.

	Spring	Summer	Autumn	Winter
No. pits (total)	6.91 ± 0.58	2.55 ± 0.53	1.21 ± 0.31	5.38 ± 0.56
No. fresh pits	1.75 ± 0.24	0.68 ± 0.30	0.42 ± 0.20	2.18 ± 0.31
No. old pits	5.14 ± 0.46	1.87 ± 0.45	0.79 ± 0.22	3.20 ± 0.36
No. faeces (total)	5.43 ± 0.54	2.32 ± 0.54	3.00 ± 0.93	4.56 ± 0.68
No. fresh faeces	1.44 ± 0.22	0.60 ± 0.22	1.04 ± 0.24	2.10 ± 0.34
No. old faeces	3.99 ± 0.41	1.72 ± 0.47	1.96 ± 0.81	2.46 ± 0.40
Proportion of faeces in pits	0.98 ± 0.01	0.70 ± 0.13	0.63 ± 0.10	0.92 ± 0.03
No. anal gland secretions (total)	0.63 ± 0.14	0.10 ± 0.11	0.09 ± 0.10	0.59 ± 0.15
Scratching	0.10 ± 0.06	0	0	0.21 ± 0.07

d.f.=3,36, $p>0.05$). The number of pits per latrine (total and old) were significantly greater in spring than during any other season.

5.3.6 Seasonal comparisons of activity between boundary and hinterland latrines

During the spring season, activity between boundary and hinterland latrines varied very little. In particular, the number of fresh faeces at boundary latrines was only marginally higher than for hinterland latrines (one-way ANOVA, $F=0.12$, d.f.=1,56, $p>0.70$), while the number of anal gland secretions per boundary latrine was actually less than the mean number for hinterland latrines, although not significant (Kruskal-Wallis, $H=0.22$, d.f.=1, $p>0.60$) (see Tables 5.3 and 5.4). The behaviour of scratching was more commonly observed at boundary latrines during this season.

A different pattern of badger behaviour between these types of latrine was detected during the summer period. Boundary latrines contained on average over twice as many fresh faeces per latrine than hinterland latrines, although this was not quite significant (one-way ANOVA, $F=2.95$, d.f.=1,29, $p=0.10$). As a result of this greater defecation on boundary latrines, one-way analysis of variance revealed a significantly larger number of faeces (total) at boundary latrines ($F=4.70$, d.f.=1,29, $p<0.05$). Despite higher levels of dunging behaviour at boundary latrines during the autumn season, no significant difference was detected in the number of fresh faeces (one-way ANOVA, $F=0.70$, d.f.=1,32, $p>0.05$), nor in the total number of faeces between the two types of latrine (one-way ANOVA, $F=1.40$, d.f.=1,32, $p>0.05$).

A reversal in the dunging behaviour was detected in the winter, with hinterland latrines containing more fresh/total faeces than boundary latrines, although not significant in either case. Scratching was more frequent at boundary

latrines over this period, although this trend was not quite significant (Kruskal-Wallis, $H=3.28$, d.f. $=1$, $p=0.07$).

5.3.7 Seasonal comparisons of activity between pasture and woodland latrines

Few major differences in activity between pasture and woodland latrines were detected throughout the year. The digging of pits in the spring occurred more frequently at latrines within woodland, although not significantly so, while faecal numbers varied little between latrines from these two habitats during this season.

In the summer, the most pronounced difference detected between the two latrine types was the larger number of fresh faeces at latrines on pasture (one-way ANOVA, $F=3.05$, d.f. $=1,27$, $p=0.09$) (Table 5.7). During the autumn, badgers seldom dug pits at latrines on pasture (one-way ANOVA, $F=5.06$, d.f. $=1,29$, $p<0.05$), however the number of fresh faeces (one-way ANOVA, $F=4.57$, d.f. $=1,29$, $p<0.05$) and total faecal numbers (one-way ANOVA, $F=8.53$, d.f. $=1,29$, $p<0.01$) were significantly greater at latrines on pasture (Table 5.8). As a result, significantly more faeces at latrines were deposited directly on to the surface of the pasture and not in pits (one-way ANOVA, $F=4.82$, d.f. $=1,29$, $p<0.05$). No significant differences were detected during the winter period between pasture and woodland latrines for any of the activities recorded.

5.3.8 Seasonal pattern of latrine visits in different habitats

The mean number of woodland latrines visited (weighted according to the availability of these latrines) per badger per night reached a peak in the spring (Table 5.9), declining through the year, before rising slightly in the winter (Kruskal-Wallis, $H=8.82$, d.f. $=3$, $p<0.05$), although no season was significantly distinct. Visits to pasture latrines reached a peak during the summer period of 2.00

Table 5.7 Comparisons between pattern of use of pasture and woodland latrines during spring (above) and summer (below); with the exception of scratching (mean score per latrine ± s.e.), figures are the mean no. per latrine ± s.e.

	Pasture latrines	Woodland latrines	F	H	P
No. pits (total)	5.35 ± 0.63	6.91 ± 0.58	2.33		n.s.
	2.50 ± 0.72	2.55 ± 0.53	0.00		n.s.
No. fresh pits	1.07 ± 0.36	1.75 ± 0.24	2.35		n.s.
	0.74 ± 0.39	0.68 ± 0.30	0.01		n.s.
No. old pits	4.29 ± 0.62	5.14 ± 0.46	1.05		n.s.
	1.76 ± 0.43	1.87 ± 0.45	0.03		n.s.
No. faeces (total)	6.62 ± 0.82	5.43 ± 0.54	1.44		n.s.
	3.63 ± 0.66	2.32 ± 0.54	1.83		n.s.
No. fresh faeces	1.25 ± 0.29	1.44 ± 0.22	0.21		n.s.
	1.66 ± 0.43	0.60 ± 0.22	3.05		n.s.
No. old faeces	5.37 ± 0.78	3.99 ± 0.41	2.90		n.s.
	1.97 ± 0.42	1.72 ± 0.47	0.15		n.s.
No. anal gland	0.45 ± 0.24	0.63 ± 0.14		1.38	n.s.
secretions	0.50 ± 0.16	0.10 ± 0.11		3.13	n.s.
Scratching	0	0.10 ± 0.06		1.59	n.s.
	0	0		-	-

Table 5.8 Comparisons between pattern of use of pasture and woodland latrines during autumn (above) and winter (below); with the exception of scratching (mean score per latrine ± s.e.), figures are the mean no. per latrine ± s.e.

	Pasture latrines	Woodland latrines	F	H	P
No. pits (total)	0.70 ± 0.25	1.23 ± 0.34	1.69		n.s.
	5.63 ± 0.93	5.38 ± 0.56	0.03		n.s.
No. fresh pits	0.08 ± 0.06	0.45 ± 0.22	5.06		<0.05
	2.94 ± 0.76	2.18 ± 0.31	0.94		n.s.
No. old pits	0.63 ± 0.24	0.77 ± 0.24	0.17		n.s.
	2.69 ± 0.66	3.20 ± 0.36	0.33		n.s.
No. faeces (total)	8.85 ± 1.64	2.36 ± 0.71	8.53		<0.01
	2.69 ± 0.85	4.56 ± 0.68	1.30		n.s.
No. fresh faeces	2.38 ± 0.45	1.05 ± 0.27	4.57		<0.05
	0.88 ± 0.37	2.10 ± 0.34	2.25		n.s.
No. old faeces	6.48 ± 1.45	1.32 ± 0.50	7.01		0.01
	1.81 ± 0.67	2.46 ± 0.40	0.44		n.s.
No. anal gland secretions	0.18 ± 0.09	0.09 ± 0.10		0.55	n.s.
	0.50 ± 0.29	0.59 ± 0.15		0.00	n.s.
Scratching	0	0		-	-
	0.13 ± 0.13	0.21 ± 0.07		0.22	n.s.

Table 5.9 Seasonal variation in number of latrine visits (weighted according to latrine availability) by badgers in different habitat types; figures are the mean number of different latrines visited per badger per night ± s.e.

	Spring	Summer	Autumn	Winter
Pasture	1.29 ± 0.35	2.00 ± 0.71	1.75 ± 0.84	0.30 ± 0.13
Woodland	5.51 ± 0.78	4.65 ± 0.89	3.05 ± 0.53	3.80 ± 0.79
Arable	0.88 ± 0.47	0.69 ± 0.38	1.49 ± 0.47	1.63 ± 0.80

per badger per night, dropping to only 0.30 during the winter ($H=8.12$, d.f.$=3$, $p<0.05$), although no season was statistically distinct. Latrines on arable land were visited relatively infrequently throughout the year, with a non significant peak over the autumn and winter period.

Analysis of the distribution of latrine visits between the different habitat types within seasons (Table 5.9) revealed that during spring (Friedmans, $F=23.29$, d.f.$=2,38$, $p=0.0001$, Tukey's HSD), autumn (Friedmans, $F=9.50$, d.f.$=2,32$, $p<0.001$, Tukey's HSD) and winter (Friedmans, $F=12.00$, d.f.$=2,38$, $p=0.0001$, Tukey's HSD), badgers visited significantly more latrines within woodland than on pasture or arable land. During summer the number of visits to woodland and pasture latrines were not significantly different, although were both significantly greater than for visits to arable latrines (Friedmans, $F=10.55$, d.f.$=2,32$, $p<0.001$, Tukey's HSD).

For each habitat type, the expected pooled mean number of latrine visits was calculated seasonally, based on the availability of latrines within each habitat. Comparing observed and expected number of latrine visits for each habitat, revealed that for spring ($X^2=19.18$, d.f.$=2$, $p<0.001$), summer ($X^2=12.17$, d.f.$=2$, $p<0.005$) and winter ($X^2=16.18$, d.f.$=2$, $p<0.001$), badgers visited more latrines in woodland than expected and fewer than expected in both pasture and arable land (Table 5.10). During the autumn season, no significant difference was detected for the different habitats ($X^2=0.47$, d.f.$=2$, $p>0.05$).

5.3.9 Expected number of defecations at latrines on pasture and woodland

For each season the expected number of defecations at latrines on pasture and woodland was calculated, based on the availability of these two types of latrine (Table 5.11). Comparing observed number of defecations with expected in spring ($X^2=2.05$), summer ($X^2=0.34$) and autumn ($X^2=2.08$), no significant differences

Table 5.10 Expected pattern of latrine visits in different habitats (bottom values), based on the availability of latrines within those habitats, in relation to that actually observed (top values); figures are pooled mean no. of latrine visits per night.

	Spring	Summer	Autumn	Winter
Pasture	21.40	34.00	29.80	6.00
	34.23	37.11	27.96	19.00
Woodland	46.60	20.00	19.50	43.50
	28.59	10.80	19.62	29.19
Arable	3.60	3.00	6.50	4.50
	8.78	9.08	8.22	5.81

Table 5.11 Expected number of defecations at latrines in pasture and woodland (bottom values), based on the availability of latrines within those habitats, in relation to that actually observed (top values); figures are pooled mean no. of defecations at latrines per night (due to small values, arable latrine defecations have been omitted).

	Spring	Summer	Autumn	Winter
Pasture	7.00	6.00	8.50	1.00
	9.99	6.69	6.19	4.47
Woodland	10.80	2.50	2.00	10.00
	7.81	1.81	4.31	6.53

were detected (in all cases, d.f. = 1, $p > 0.05$). However, during the winter season, a significant difference was found, with badgers defecating more than expected at woodland latrines and less than expected at latrines on pasture ($X^2 = 4.60$, d.f. = 1, $p < 0.05$).

5.3.10 Linear habitat features and latrine distribution

The number of hinterland latrines belonging to the five study groups, against particular types of field boundary, were compared to the expected number based on the availability of the different boundaries (total length within the five territories). Latrines included those located on pasture, woodland and arable land. Analysis revealed an overall significant difference between the observed number of latrines against particular boundaries and that expected ($X^2 = 17.68$, d.f. = 6, $p < 0.01$) (Table 5.12). In each case those boundaries which restricted the badgers movements (sheep netting, hedgerow, combined hedgerow and thick undergrowth) had a greater number of latrines beside them than expected. Likewise, those boundaries that did not restrict movement (barbed wire, walls with gates and open woodland) each had a lower number of latrines against them than expected.

5.3.11 Habitat and latrine distribution

In Table 5.13 expected numbers of latrines in different habitats were calculated based on the total area of each habitat covering the five social groups. For these five groups 51.04% of all latrines were located in woodland, with woodland comprising only 16.67% of the total area. As revealed in Table 5.13, greater numbers of latrines were located in woodland than expected, while fewer than expected were found on pasture and arable land ($X^2 = 21.58$, d.f. = 2, $p < 0.001$).

Table 5.12 Total number of hinterland latrines in the five study groups against particular types of field boundary; combined hedges are those in conjunction with either barbed wire, sheep netting or a wall. Latrines were located in either pasture, woodland or arable land and their positions established from bait marking in February 1991.

Field boundary type	Observed number of latrines	Expected number of latrines	Partial X^2 values
Sheep netting	11	5.76	4.77
Thick undergrowth	5	2.58	2.27
Combined hedgerow	13	10.00	0.90
Hedgerow	7	5.64	0.33
Open woodland	0	7.15	7.15
Barbed wire	2	5.15	1.93
Wall	7	8.70	0.33

Total $X^2 = 17.68$

d.f. $= 6$

$p < 0.01$

Table 5.13 Distribution of latrines between the major habitat types in 1991; expected numbers have been calculated based on the total area of each habitat covering the five social groups. Figures in brackets beside habitats are the percentage that each habitat contributes to the total area; other bracketed figures are the percentage of total latrines.

Habitat type	Observed no. of latrines	Expected no. of latrines	Partial X^2 values
Woodland (16.67)	98 (51.04)	67.3	14.00
Pasture (68.28)	81 (42.19)	108.3	6.88
Arable (9.95)	13 (6.77)	16.4	0.70

Total $X^2=21.58$

d.f.$=2$

$p<0.001$

5.4 Discussion

Several studies have suggested that latrine use reflects territorial behaviour (Kruuk 1978; Cheeseman *et al.* 1981; Roper, Shepherdson & Davies 1986). Clearly then, if badgers are restricting access to oestrous sows (Roper, Shepherdson & Davies 1986) or defending food resources (Kruuk 1978) it should be expected that latrine activity will coincide with either the breeding season or a time when food availability is at its lowest. If badgers defend food resources then defence may not necessarily coincide with a period when food availability is at its lowest but when competition for food is greatest.

Latrine use has been reported to show a seasonal peak in early spring (Neal 1977; Kruuk 1978) and later by Roper, Shepherdson & Davies (1986) who examined how the pattern of latrine use varied on a monthly basis throughout the year. The spring peak reported by Roper, Shepherdson & Davies (1986) in the number of pits per latrine was supported by the present study, although contrary to the secondary peak during the autumn, believed to be due to an upsurge in mating activity at that time, this study found very low levels of pit digging during this season, especially at latrines on pasture. Another measure of latrine activity used by Roper, Shepherdson & Davies (1986) was based on the number of pits containing fresh faeces. Clearly this will depend largely on the pit digging behaviour of badgers and does not reflect seasonal changes in dunging behaviour. Even with pits available to badgers at latrines during the autumn, faeces were frequently deposited directly on to the surface of the ground away from pits. This behaviour was also recorded in the summer period, although not as marked. Certainly during the present study recording only pits containing fresh faeces would have been misleading considering the above observations and that active latrines can contain few or no pits at all.

In relation to faecal output, the distribution of faeces on a badger's territory is probably a more significant measure of territoriality than seasonal patterns of faecal output, which is likely to be affected by variation in food input and probably more importantly by variation in group size throughout the year. Summer was the only season in which faecal numbers differed significantly between boundary and hinterland latrines, with badgers defecating preferentially at boundary latrines. The difference in the percentage of boundary and hinterland latrines containing fresh faeces was also maximum during the summer, with 71.4% of boundary and only 58.8% of hinterland latrines containing fresh faeces. The percentage of latrines containing fresh faeces was also a measure of territorial activity used by Roper, Shepherdson & Davies (1986). As in the present study, they also found the overall percentage of latrines containing fresh faeces was at its lowest during the summer months, which they used as evidence to suggest a lower level of territoriality at that time. However, as the above values indicate, badgers were largely ignoring hinterland latrines and defecating on the boundary, resulting in a large percentage of inactive hinterland latrines, which when included into an overall analysis would give the impression of low latrine activity during the summer. Boundary latrine defecations reached a peak during the autumn, although were not significantly greater than for hinterland latrines. These findings suggest that during the summer and autumn months, the use of faeces in territorial demarcation is at its highest. As food availability can be at its lowest in the summer and the demand for food from within the group increases from spring to autumn due to cub growth, the peak in dunging behaviour observed at boundary latrines during the summer and autumn supports the food based hypothesis of territorial defence.

Anal gland secretion reached a peak during the winter/spring period for both boundary and hinterland latrines, supporting the data presented in Chapter 3. During the spring, a period of greatest extra-territorial movement by young adult males, only 34.78% of boundary latrines contained anal gland secretion, compared

to 45.71% of hinterland latrines. Clearly anal gland secretion has a very limited role in territorial defence and the fact that it originates primarily from adult sows, suggests that its function is to advertise the sexual condition of its producer.

Ground scratching at latrines was only observed during the winter/spring period. This behaviour was performed to a greater extent during the winter season, especially at boundary latrines. Kruuk (1978) also reported that scratching was more common at boundary latrines at this time. Although not quantified, adult males spool-and-line tracked during late winter and early spring, frequently scratched at boundary latrines. This was detected when the trail of thread through a latrine site disappeared as a result of it being covered in loose earth. As I was generally close behind the focal animal (approximately 30mins) I assumed that these activities resulted from the behaviour of the focal animal, although were clearly difficult to quantify. It has been suggested in a number of other species that ground scratching was associated with the deposition of scent from inter-digital glands (Ewer 1973; Peters 1974; Peters & Mech 1975). The behaviour of ground scratching has also been viewed as a composite signal, combining both chemical and visual components (Smith 1977; Wickler 1978), and as an intimidation display (Ewer 1968). However, the literature on ground scratching and its function in badgers is very limited.

Possibly associated with latrine scratching was the behaviour of pit digging which was also more frequent over the winter/spring period. Digging pits may be another means of dispersing scent from the inter-digital glands as during winter/spring large numbers of fresh pits at latrines contained no faeces or anal gland secretion. From Chapter 3 it was clear that badgers seldom urinated in pits at latrines, although when they did they urinated on top of faeces, either over-marking other faeces or more commonly urinating on their own faeces. Ground scratching was more common at boundary latrines during the winter/spring period, whereas

the number of fresh pits at boundary and hinterland latrines were similar during this period. The function of these pits is therefore unclear.

The scratching behaviour observed at boundary latrines during the winter/spring period would suggest that this particular behaviour was associated with the defence of oestrous females, supporting the anti-kleptogamy hypothesis. It is possible that due to the greater energetic demands during the summer and autumn, time consuming activities such as scratching and even digging are not pursued at these times due to energetic constraints. During the autumn badgers emerged early and spent greater time foraging than at any other time of the year and were frequently observed foraging before sunset and after sunrise. During winter and spring emergence times were erratic with badgers occasionally spending all night below ground. Therefore the investment of time into foraging by the group and territorial boundary patrolling by adult males (Chapter 3) during the autumn and to a lesser extent summer, are probably performed at the expense of time consuming latrine activities. This may also explain why during the autumn, 75% of urinations were made while the badgers were travelling (i.e. trails), while during the winter, 75% of urinations were performed with the badger stationary (i.e. patches) (see Chapter 4).

As reported in Chapter 1, faeces are likely to be of minor importance in the transmission of *M. bovis* from badgers to cattle. Nevertheless the presence of infected faeces at latrines on pasture would still pose a risk to cattle, particularly those cattle that appear to be unselective in their grazing behaviour. So far the significance of latrines in the process of TB transmission is largely unclear.

One of the major problems in attempting to calculate the significance of latrines in transmission, is in deciding how the number of faeces at the latrine site will affect the probability of contact between cattle and badger excreta. Given the strong avoidance of faeces by cattle, latrines heavily contaminated by faeces may be more easily avoided than latrines containing relatively small numbers of faeces.

This may also reduce the probability of contact between cattle and badger urine located at latrines. Alternatively a latrine containing large numbers of faeces is likely to pose a greater risk towards cattle that exhibit unselective grazing behaviour. As a high percentage of cattle have been shown to strongly avoid close contact with badger faeces on pasture (Benham & Broom 1991), perhaps a simpler approach of assessing the role of latrines would be to look at the risk towards those cattle that graze unselectively.

During the summer season the ratio of fresh faeces to old was approximately 1:1, whereas during the spring, latrines on pasture contained over 4x more old faeces than fresh. This rapid decomposition of faeces during the summer is likely to accelerate the destruction of *M. bovis* (Wilesmith 1991). The relatively low number of faeces observed at pasture latrines, resulting from decomposition, would also suggest that the risk of infection from latrines is relatively low during the summer.

The peak in both fresh and the total number of faeces per pasture latrine during the autumn indicates that unselective grazers are probably at greatest risk during this season. A significant behaviour during this season was that only 18% of faeces were deposited in pits, the remainder being deposited directly on to the pasture. This behaviour was almost certainly associated with the availability of pits, as digging was at an extremely low level during this season. Although pit digging was low at both woodland and pasture latrines during the autumn, latrines in woodland contained significantly more fresh pits than pasture latrines. Digging on pasture at this time of the year may have been impeded due to the longer grass length. As a consequence of this behaviour, faeces were less conspicuous and generally concealed within thick vegetation. When grass is long, cattle have been observed to eat the tops of the leaves over areas contaminated with cattle slurry (Broom, Pain & Leaver 1975; Pain & Broom 1978). This behaviour may be important in bringing cattle into close contact with excreta during this season. The

low level of scratching and digging at pasture latrines in the autumn may result in the whole latrine site being less conspicuous to cattle, resulting in greater contact with cattle and badger excretory products.

In spring the greater digging activity and the high percentage of faeces in pits (84%) may be significant in lowering the risk to cattle. Benham & Broom (1991) found no evidence to suggest that newly turned-out cattle were attracted to latrines at this time, with cattle spending significantly less time grazing latrine sites than non-latrine sites. Clearly further work is needed to examine on a seasonal basis how the contact between cattle and latrines is likely to be affected by latrine activity.

As Table 5.9 shows, the mean number of woodland latrines visited per night was greater than visits to pasture and arable latrines during each season. This was significant during spring, autumn and winter. Badgers also visited more latrines than expected in woodland and fewer than expected on pasture and arable land during spring, summer and winter. This apparent preference for visiting woodland latrines may to some extent be related to the distribution of latrines along runs in woodland. Large numbers of latrines were located along the major woodland runs radiating from the main setts. This system of runs terminated at woodland edges so that apart from the boundary run, the only badger runs in pasture and arable land were crossing point runs through field boundaries. As a consequence the density of latrines in woodland was calculated at 3.14 ha^{-1}, approximately five times that for pasture (0.64 ha^{-1}) and arable land (0.70 ha^{-1}).

Although badgers visited large numbers of woodland latrines throughout the year, faeces were deposited at latrines in different habitats in accordance with latrine availability in the different habitats, except for the winter season. During the winter badgers defecated more than expected at woodland latrines as badgers spent a large proportion of the night within woodland around the main sett. The pattern of defecation at other times of the year is not surprising since the dunging behaviour of

badgers in different habitats and the availability of latrines in different habitats are obviously closely linked as latrines are only maintained by badgers defecating at them.

The relatively high number of woodland latrines visited throughout the year is an important factor in relation to the latrine manipulations discussed in the previous chapter. Apart from woodland being generally inaccessible to cattle, this habitat also has the advantage of maximising the probability of detection of artificial latrines and/or foreign faeces placed at existing woodland latrines. Obviously with larger numbers of badgers detecting artificial latrines this would increase the likelihood of obtaining a response from badgers and possibly increase the level of dunging behaviour within woodland.

The distribution of hinterland latrines against particular field boundaries, showed a similar relationship to the selection of boundaries used for the deposition of urine on crossing point runs. The linear features which restrict badger movements to a limited number of crossing points are particularly important. Initially only hinterland latrines were included in the analysis as for the five groups only 52% of boundary latrines and 92% of hinterland latrines were located beside field boundaries. The distribution of boundary latrines around a territory is probably more closely associated with the requirements of territorial defence. It was also felt that the positioning of a boundary latrine against a particular field boundary arose primarily because that field boundary was the dividing line between territories, and did not therefore relate to the characteristics of that field boundary. When boundary latrines were included into the analyses, greater overall significance was detected, suggesting that badgers probably select linear features as territory dividers that restrict badger movement.

The reasoning behind these observations is that those field boundaries that restrict badger movement to a limited number of crossing points are selected preferentially for depositing scent markers. In this way badgers maximise the

probability of detection of their scent markers by conspecifics. These results suggest there is a close link between crossing point urination sites and latrines, and it is hypothesised that a proportion of heavily used crossing points receiving initially urine eventually form latrines. Throughout the year the only defecation from a focal badger away from a latrine was produced by an adult female in the winter on a well used crossing point run. The infrequency of this behaviour may explain why new latrine sites are only occasionally created at the study site. The significance of linear features has been shown by White, Brown & Harris (1993), who found that high risk TB areas had a greater degree of habitat heterogeneity and linear features.

In conclusion, dunging behaviour has been shown to be associated with the defence of food resources, although other latrine activities such as scratching and digging are more closely related to the defence of females during winter/spring, providing some support for the sex-based hypothesis. However, these activities may be of secondary importance in territory defence, occurring during the winter/spring period when boundary defecations were relatively low. One of the major problems of taking data directly from latrines is that it provides no indication of the participation of individual age and sex categories within the group in scent marking and territorial defence. Significant behavioural patterns in territorial defence by certain age and sex categories within the group may be missed if they are not exaggerated by a similar behaviour from other members of the group. This stresses the importance of determining the role of individuals within the group in any analyses of territoriality.

Latrines on pasture during the autumn containing large numbers of faeces deposited directly on to the pasture probably present a significant risk to cattle. The rapid decomposition of faeces seen during the summer suggests that *M. bovis* survival at this time is minimum. The higher number of latrines against linear features that restrict badger movement suggests that as with crossing point

urinations there may a positive relationship between the number of latrines in an area and the density of field boundaries.

CHAPTER 6

MODELS FOR PREDICTING LATRINE DENSITY

6.1 Introduction

Areas in the south-west of England subject to repeated badger control operations have been shown to contain greater landscape heterogeneity and a higher density of linear habitat features (White, Brown & Harris 1993). Since badgers may urinate on pasture after crossing through a linear feature, and that the number of crossing point urinations increased with the number of linear features crossed (Chapter 4), it has been hypothesised that these urinations are a major source of bovine tuberculosis infection in cattle, and that areas with increased numbers of linear features have greater levels of contamination of pasture with badger urine and hence increased opportunities for disease transmission.

Using logistic regressions (White, Brown & Harris 1993) were able to distinguish between random and control operation squares with up to 75% accuracy based simply on map-derived habitat features. Although crossing point urinations are associated with linear features it was obviously not possible to quantify the potential risk from these urinations outside the Cotswold study area, and so data on the distribution of latrines recorded from badger control operations by the Ministry of Agriculture, Fisheries and Food were used in these analyses. It was hoped that by analysing map derived habitat variables it would be possible to predict the density of latrines within a particular area and from this access the potential risk to cattle on different landscape types.

Preliminary evidence suggests that the pattern of resource distribution in a given habitat and the influence of that pattern on the social organisation of a

particular species, may underlie some of the features of the distribution of latrines. Also the pattern of latrine distribution by different species may be explicable in terms of type and quality of resources to be defended, although as yet insufficient data are available to test this hypothesis (Macdonald 1980). Examining which factors play a role in determining the density of both boundary and hinterland latrines should provide a better understanding of the mechanisms involved in territoriality in the badger.

6.2 Methods

Data from the study site on the Cotswold escarpment in Gloucestershire formed the basis of these analyses, with these baseline data being used to predict latrine densities in four areas (Cornwall, Staffordshire, Gloucestershire and Avon). Latrine densities were derived from bait-marking data supplied by the Ministry of Agriculture, Fisheries and Food from these four counties. Latrine densities were calculated for individual social groups. A bit pad was used to determine territory size, territory boundary length, field boundary length, area of woodland and the combined area of pasture and arable land within territories. This enabled the density of latrines within different habitats to be calculated. Data on social group sizes at the Cotswold study area were also supplied by the Ministry of Agriculture, Fisheries and Food. Social group sizes were determined from capture records at the end of each year. Animals found dead after the period of bait-marking were included in the analyses, although since cubs were not involved in scent marking at latrines during the period of bait marking, this category was excluded from the analyses. Group size was therefore a measure of the number of adults and yearlings within the group. Social group sizes for Cornwall, Staffordshire and Avon were obtained from published data (Cheeseman *et al.* 1981; Cheeseman *et al.* 1985), and

from the other site in Gloucestershire from unpublished data supplied by the Ministry of Agriculture, Fisheries and Food.

Data from the Cotswold escarpment study site were analysed for 1991 and 1992, with group sizes, territory sizes and latrine densities averaged for the two years. Thirty social groups were included in these analyses. Four groups were excluded since large areas of settlement occurred within these territories, and the disturbance may have affected latrine numbers or their distribution.

All active latrines located during the bait-marking survey were included in the analyses. This included latrines that did not contain plastic pellets. Analysing the data by social groups posed problems when calculating boundary latrine densities, since these latrines were shared by two and even three or four different groups. For the density calculations, when summing the number of boundary latrines for a particular group, a boundary latrine shared by another group was counted as 0.5. The density of latrines on pasture and arable land on badger territories was calculated by two different methods. The first determined the number of latrines on pasture and arable land km^{-2} of pasture and arable land and has been referred to as the density of latrines on pasture and arable land. The second method involved calculating the number of latrines on pasture and arable land km^{-2} of territory.

6.2.1 Study areas

Habitat details for the Cotswold escarpment study site are given in Chapter 1. The area in Cornwall was fairly isolated, surrounded on three sides by a river estuary, and supported six social groups of badgers. Hills rose to 76m above sea-level with the slopes from the estuary covered mainly by mature deciduous woodland. Most of the setts were located in these belts of woodland which comprised 6% of the total area. Pasture and arable land covered 80% and 9% of

the area respectively, while settlement covered a further 5%. Fields were generally smaller than at the study site in Gloucestershire, with field boundaries typically formed from hedge-banks (earth covered stone walls with vegetation growing on top). Farming in the area was mainly beef and dairy with some sheep.

The area in Avon was relatively hilly, and ranged between 48m and 233m above sea-level. The area contained seven social groups, with most of the setts located in hill sandstone strata amongst deciduous woodland. The area comprised of 1% settlement, 7% woodland, 79% permanent pasture and 13% arable land. Farming was mainly beef and dairy.

The Staffordshire area supported five social groups, with a hilly terrain that ranged between 150 and 280m above sea-level. Steep sided valleys were dominated by deciduous and mixed woodland and comprised 15% of the total area. The remainder of the area was predominantly permanent pasture. Field sizes were small (5-15 ha), with field boundaries of hedges or dry stone walls. Farming was mainly beef and dairy with some sheep. The Gloucestershire area contained five social groups. Hills rose to 276m above sea-level, and woodland covered 35% of the total area.

6.2.2 Methods of population estimate

On the Cotswold escarpment badgers were caught exclusively in cage traps (Cheeseman & Mallinson 1980). In Avon badgers were caught in June 1979 predominantly in cage traps, although snares were also used to catch trap-shy individuals. At the site in Cornwall only snares were used for the removal of badgers. About 50 snares were deployed around setts on each of 18 nights in October and November 1978. In Staffordshire saturation trapping with both cage traps and snares was used to achieve the optimal rate of removal. Trapping began in June 1982. At the Gloucestershire site badgers were caught mainly in cage traps

although snares were also used. Trapping commenced in the middle of June 1982 and continued for a period of 16 nights.

6.3 Results

6.3.1 Relationship between latrine density and the density of linear habitat features

No significant relationship was found between the density of latrines on a territory (no. km^{-2}) and field boundary density (Figure 6.1) ($F=0.26$, $R^2=0$, $d.f.=1,28$, $p>0.05$). Similarly boundary (Figure 6.2) and hinterland (Figure 6.3) latrine densities showed no correlation with field boundary density, although the density of latrines on pasture and arable land was significantly correlated with field boundary density (Figure 6.4) ($F=4.53$, $R^2=10.8$, $d.f.=1,28$, $p<0.05$). When latrines on pasture and arable land were separated into hinterland and boundary latrines, neither type of latrine were found to be correlated with field boundary density.

6.3.2 Relationship between latrine density and group size

Group size was found to be significantly correlated with the density of latrines on badger territories (Figure 6.5) ($F=7.61$, $R^2=18.6$, $d.f.=1,28$, $p=0.01$). Analysing boundary and hinterland latrines separately revealed that boundary latrine density was not correlated to group size ($F=0.11$, $R^2=0$, $d.f.=1,28$, $p>0.05$), although as shown in Figure 6.6 the density of hinterland latrines within territories was found to be correlated with group size ($F=13.25$, $R^2=29.7$, $d.f.=1,28$, $p=0.001$). Group size was not correlated with the density of latrines on pasture and arable land ($F=2.32$, $R^2=4.3$, $d.f.=1,28$, $p>0.05$), nor with hinterland latrine density on pasture and arable ($F=2.73$, $R^2=5.6$, $d.f.=1,28$,

Figure 6.1 Relationship between the number of latrines km^{-2} and field boundary density (m km^{-2}) ($F=0.26$, $R^2=0$, d.f. $=1,28$, $p>0.05$).

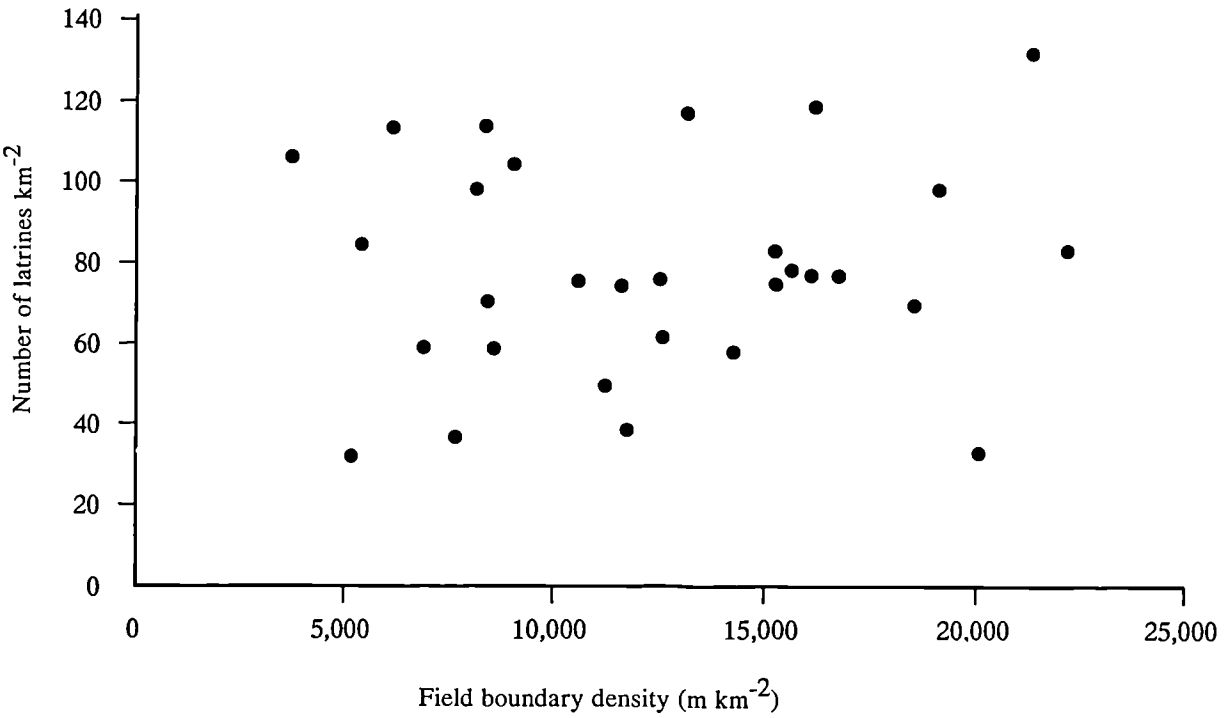


Figure 6.2 Relationship between the number of boundary latrines km^{-2} and field boundary density (m km^{-2}) ($F=3.27$, $R^2=7.3$, d.f. $=1,28$, $p>0.05$).

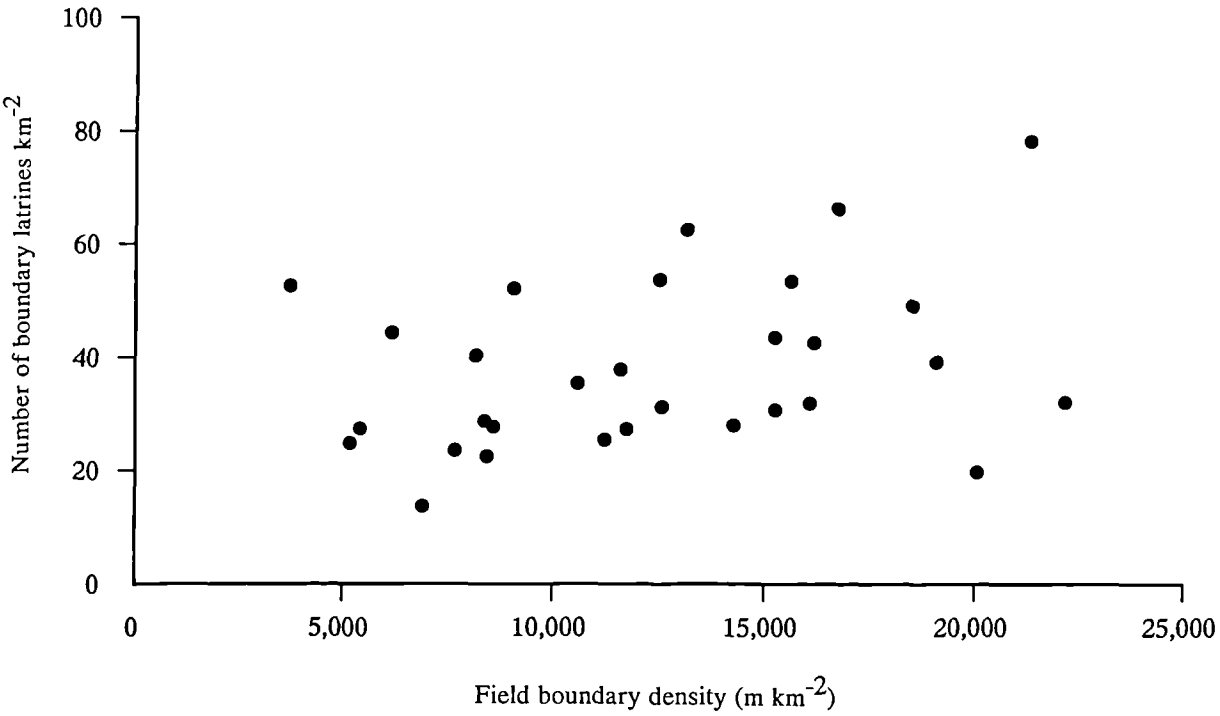


Figure 6.3 Relationship between the number of hinterland latrines km^{-2} and field boundary density (m km^{-2}) ($F=0.36$, $R^2=0$, d.f. $=1,28$, $p>0.05$).

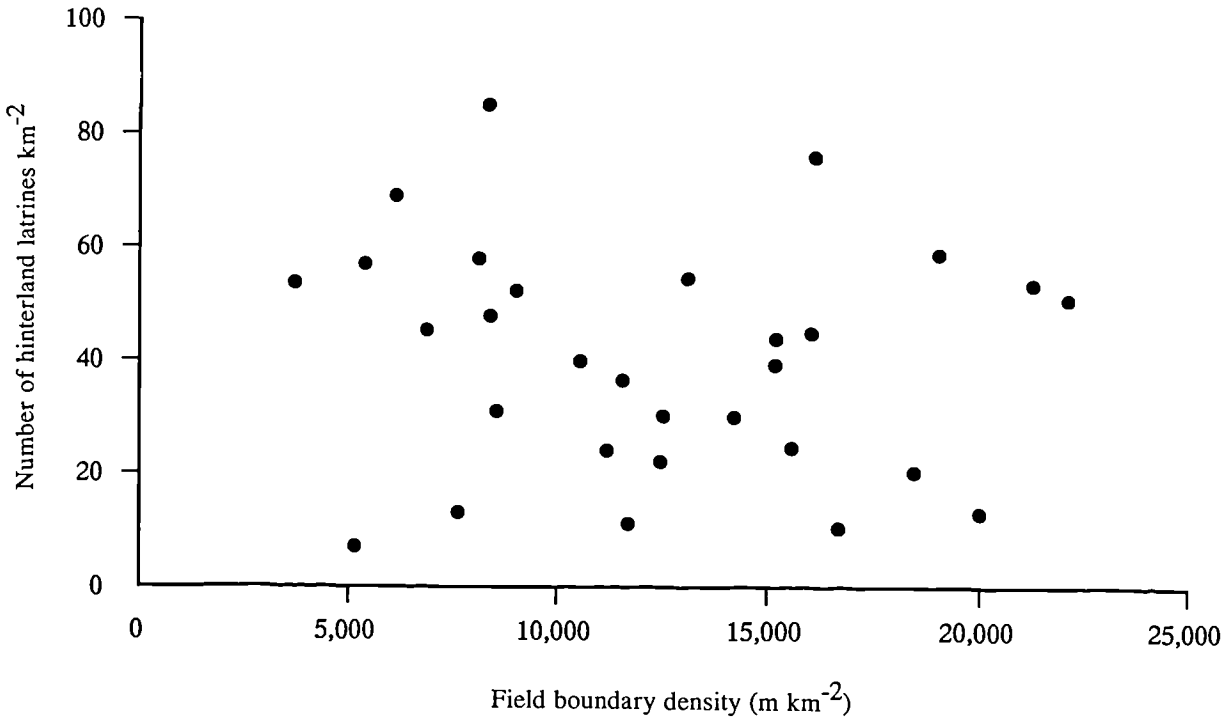


Figure 6.4 Relationship between the density of latrines on pasture and arable land (number km^{-2} of pasture and arable land) and field boundary density (m km^{-2}) ($F=4.53$, $R^2=10.8$, d.f.$=1,28$, $p<0.05$).

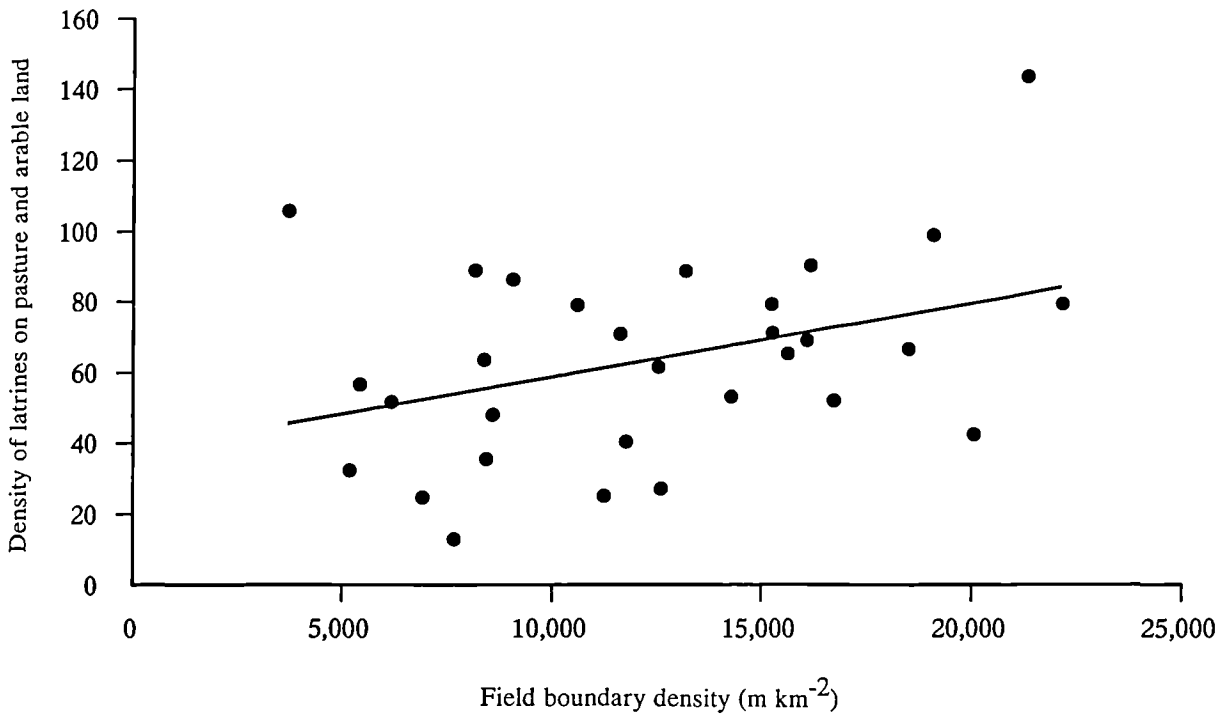


Figure 6.5 Relationship between the number of latrines km^{-2} and group size (number of adults and yearlings) ($F=7.61$, $R^2 \approx 18.6$, d.f. $=1,28$, $p=0.01$).

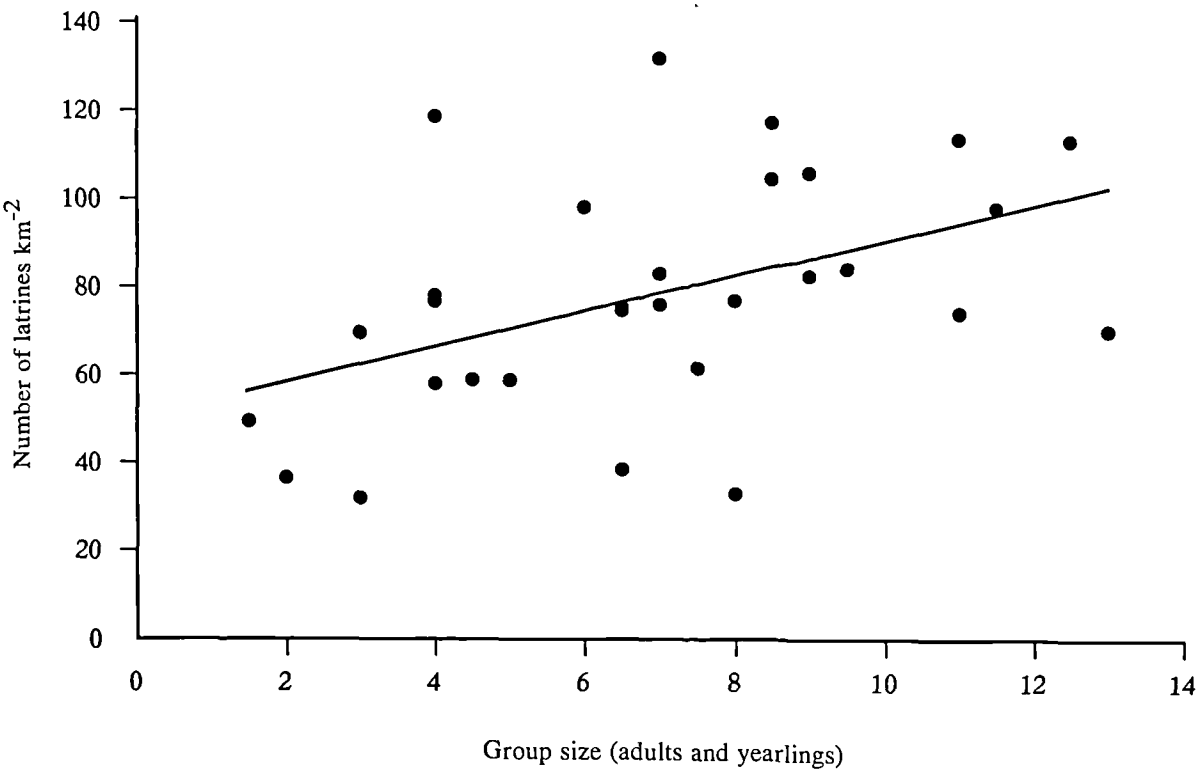
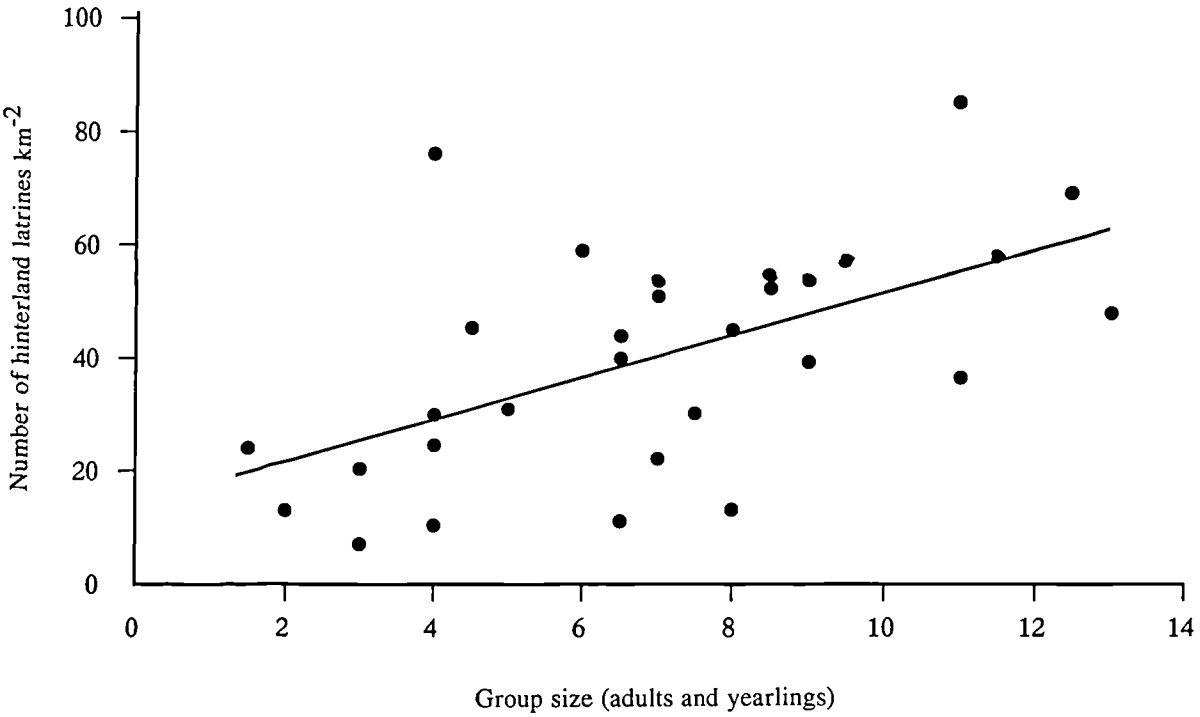


Figure 6.6 Relationship between the number of hinterland latrines km^{-2} and group size (number of adults and yearlings) ($F=13.25$, $R^2=29.7$, d.f.$=1,28$, $p=0.001$).



$p>0.05$) and boundary latrine density on pasture and arable land ($F=0.79$, $R^2=0$, d.f.$=1,28$, $p>0.05$).

6.3.3 Relationship between latrine density and territory size

The density of all latrine types grouped was not correlated with territory size ($F=4.03$, $R^2=9.5$, d.f.$=1,28$, $p>0.05$). The number of hinterland latrines increased significantly with increased territory size ($F=15.39$, $R^2=33.2$, d.f.$=1,28$, $p=0.001$). Although group size and territory size were not related, the number of hinterland latrines on a territory was controlled for group size by calculating the number of hinterland latrines per badger (Figure 6.7). This revealed that the number of hinterland latrines per badger increased significantly with increased territory size ($F=7.30$, $R^2=17.8$, d.f.$=1,28$, $p<0.05$). The number of hinterland latrines per badger was also found to be significantly correlated with the length of territory boundary per badger (Figure 6.8) ($F=13.45$, $R^2=30.3$, d.f.$=1,28$, $p=0.001$), although no correlation was found between the length of territory boundary per individual and territory size ($F=1.17$, $R^2=0$, d.f.$=1,28$, $p>0.05$). Due to the increase in hinterland latrine numbers with increased territory size, the density of hinterland latrines was not correlated with territory size ($F=0.01$, $R^2=0$, d.f.$=1,28$, $p>0.05$).

Boundary latrine numbers remained fairly constant across the range of territory sizes in the analyses. This was apparent from Figure 6.9, which showed that the mean distance between boundary latrines was positively correlated with territory size ($F=12.13$, $R^2=28.4$, d.f.$=1,27$, $p=0.002$). Boundary latrine density showed a significant negative correlation with territory size ($F=21.02$, $R^2=40.8$, d.f.$=1,28$, $p<0.0001$), and was found to show a greater correlation with territory size after a logarithmic transformation of boundary latrine density (Figure 6.10) ($F=26.83$, $R^2=47.6$, d.f.$=1,28$, $p<0.0001$). The overall density of

Figure 6.7 Relationship between the number of hinterland latrines per badger and territory size (km^2) ($F=7.30$, $R^2=17.8$, d.f. $=1,28$, $p<0.05$).

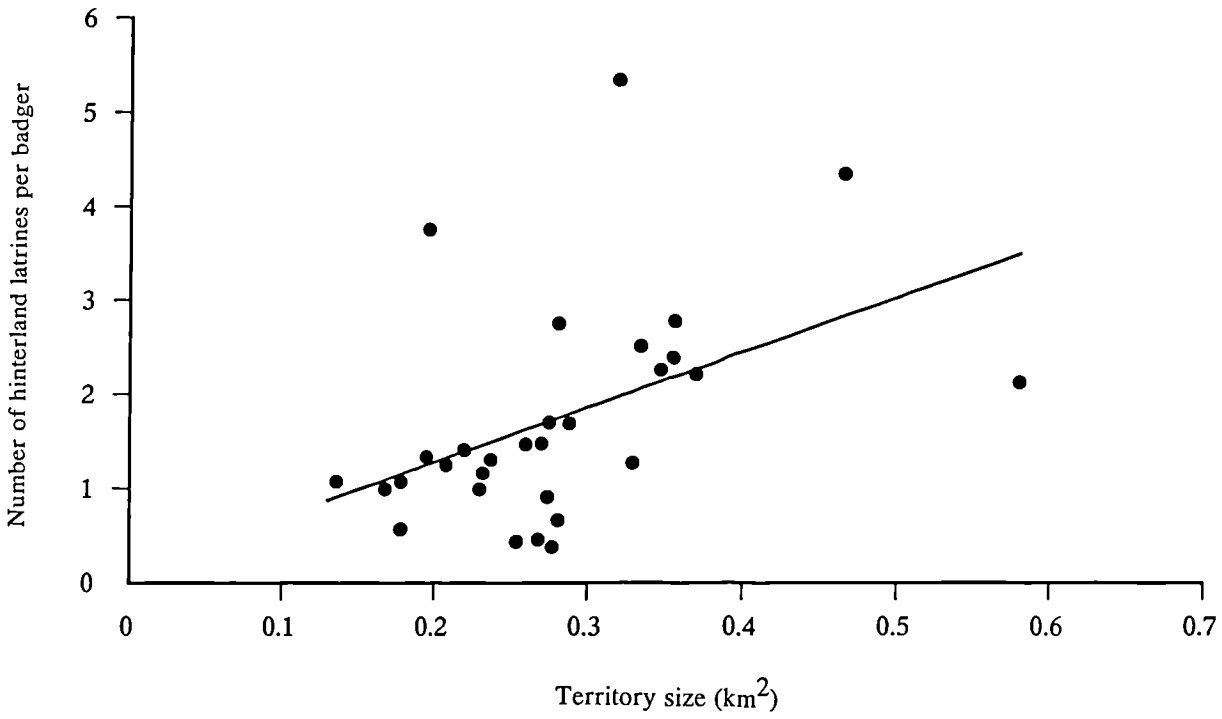


Figure 6.8 Relationship between the number of hinterland latrines per badger and the length of territory boundary per badger (m) ($F=13.45$, $R^2=30.3$, d.f. $=1,28$, $p=0.001$).

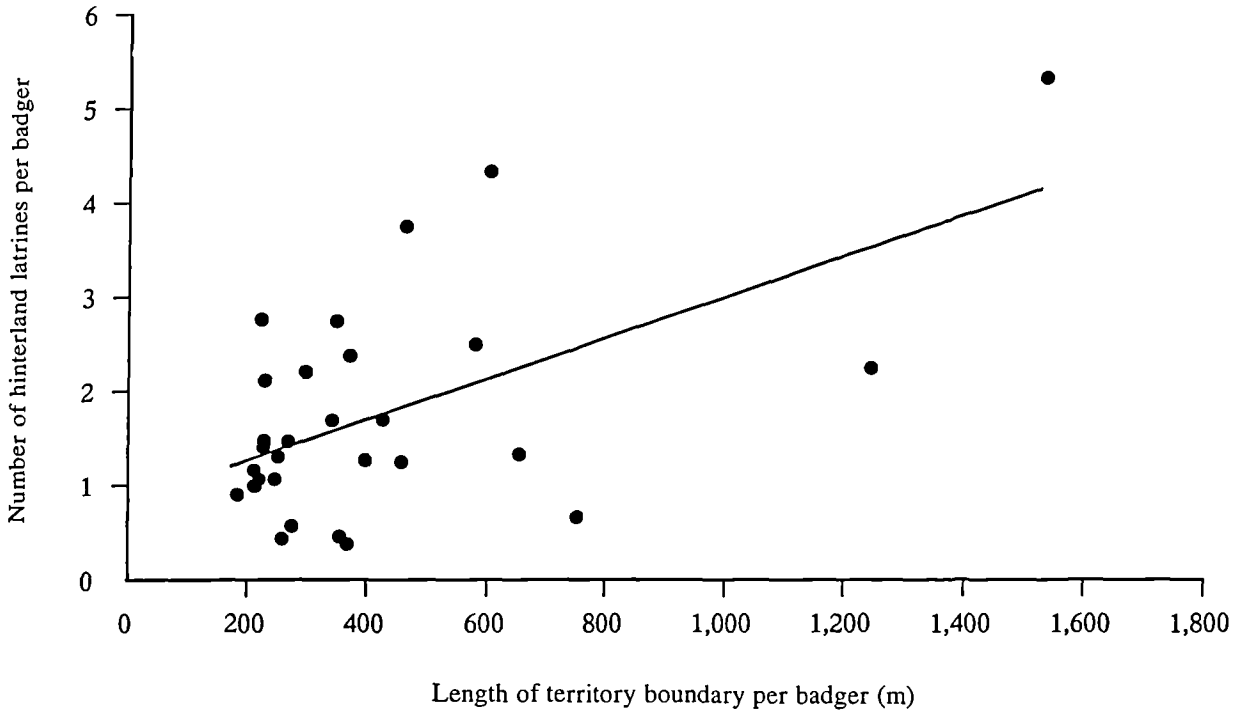


Figure 6.9 Relationship between inter-boundary latrine distance (m) and territory size (km^2) ($F=12.13$, $R^2=28.4$, d.f.$=1,27$, $p\approx0.002$).

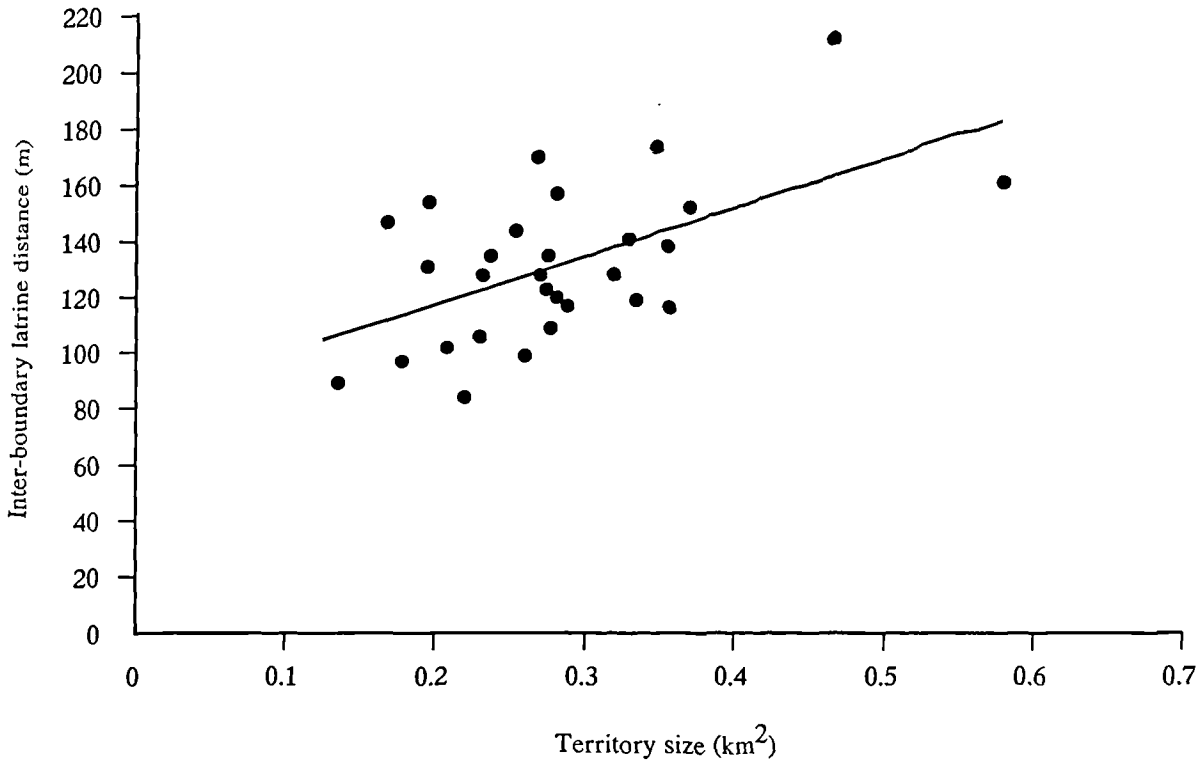
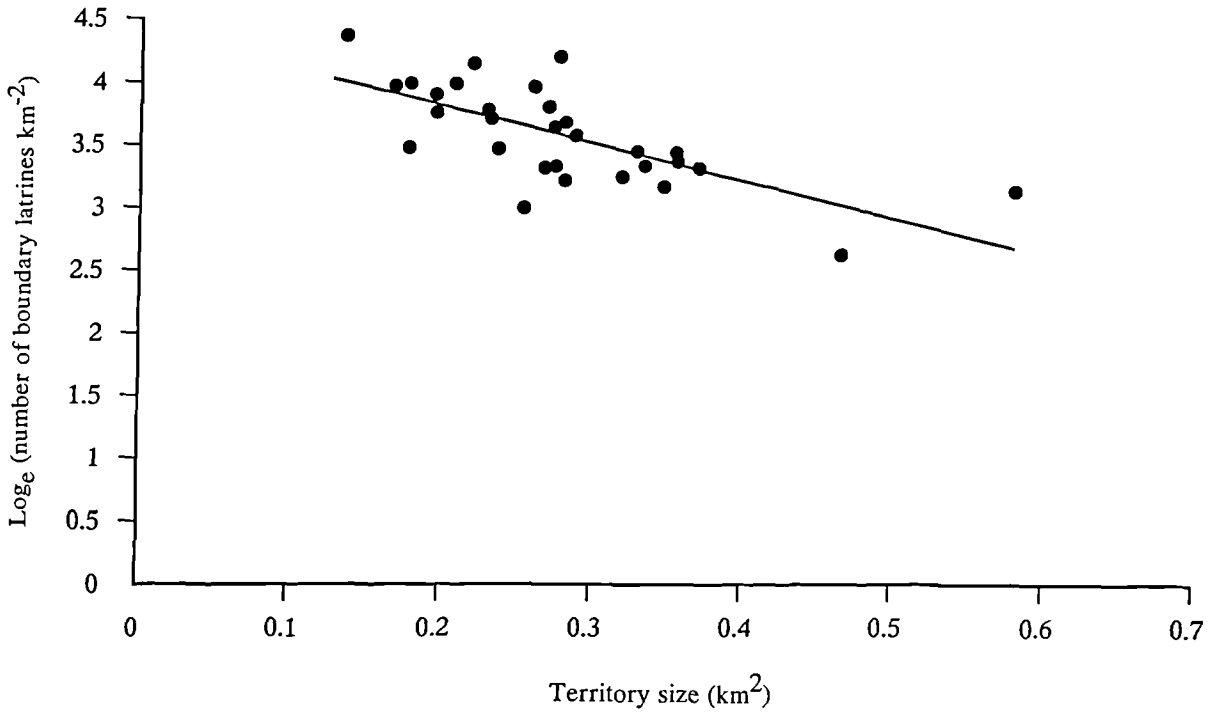


Figure 6.10 Relationship between $\log_e$ (number of boundary latrines km^{-2}) and territory size (km^2) ($F=26.83$, $R^2=47.6$, d.f.$=1,28$, $p<0.0001$).



latrines on pasture and arable land (Figure 6.11), was also found to be negatively correlated with territory size ($F=15.53$, $R^2=33.6$, d.f.$=1,28$, $p<0.0001$). This relationship resulted entirely from the negative correlation found between boundary latrine density on pasture and arable land with territory size ($F=28.18$, $R^2=48.4$, d.f.$=1,28$, $p<0.0001$) and not from hinterland latrine density on pasture and arable land with territory size ($F=2.20$, $R^2=4.0$, d.f.$=1,28$, $p>0.05$).

6.3.4 The influence of woodland on latrine density

Although in Chapter 5 it was found that woodland was selected preferentially for latrine sites, no correlation was detected between latrine density on a territory and the proportion of woodland within the territory ($F=0.09$, $R^2=0$, d.f.$=1,28$, $p>0.05$). Likewise no correlation was found between the density of boundary latrines ($F=1.47$, $R^2=1.6$, d.f.$=1,28$, $p>0.05$), hinterland latrines ($F=1.73$, $R^2=2.4$, d.f.$=1,28$, $p>0.05$) and in particular the density of latrines on pasture and arable land (Figure 6.12) with the proportion of woodland within territories ($F=0.14$, $R^2=0$, d.f.$=1,28$, $p>0.05$).

6.3.5 Analysis of test data

For the site in Cornwall, field boundary density explained 71.0% of the variation in the density of latrines on pasture and arable land ($F=13.21$, $R^2=71.0$, d.f.$=1,4$, $p<0.05$). A similar pattern existed between latrine density on pasture and arable and field boundary density for the Avon site ($F=13.74$, $R^2=68.0$, d.f.$=1,5$, $p<0.02$). However, for the Gloucestershire and Staffordshire sites field boundary density was not correlated with pasture and arable latrine density. At the site in Cornwall ($F=9.79$, $R^2=63.8$, d.f.$=1,4$, $p<0.05$) and for the area in Avon ($F=7.31$, $R^2=51.3$, d.f.$=1,5$, $p<0.05$), latrine density on pasture and arable land

Figure 6.11 Relationship between $\log_e$ (density of latrines on pasture and arable land) and territory size (km^2) ($F=15.53$, $R^2=33.6$, d.f. $=1,28$, $p<0.0001$).

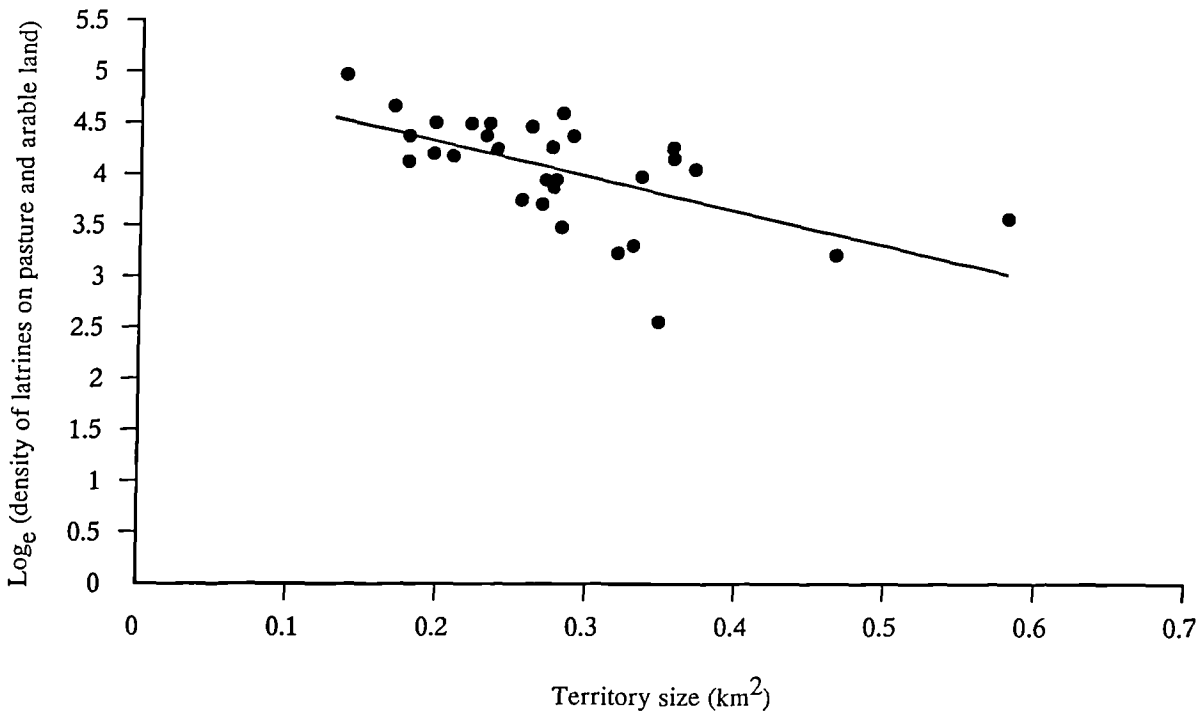
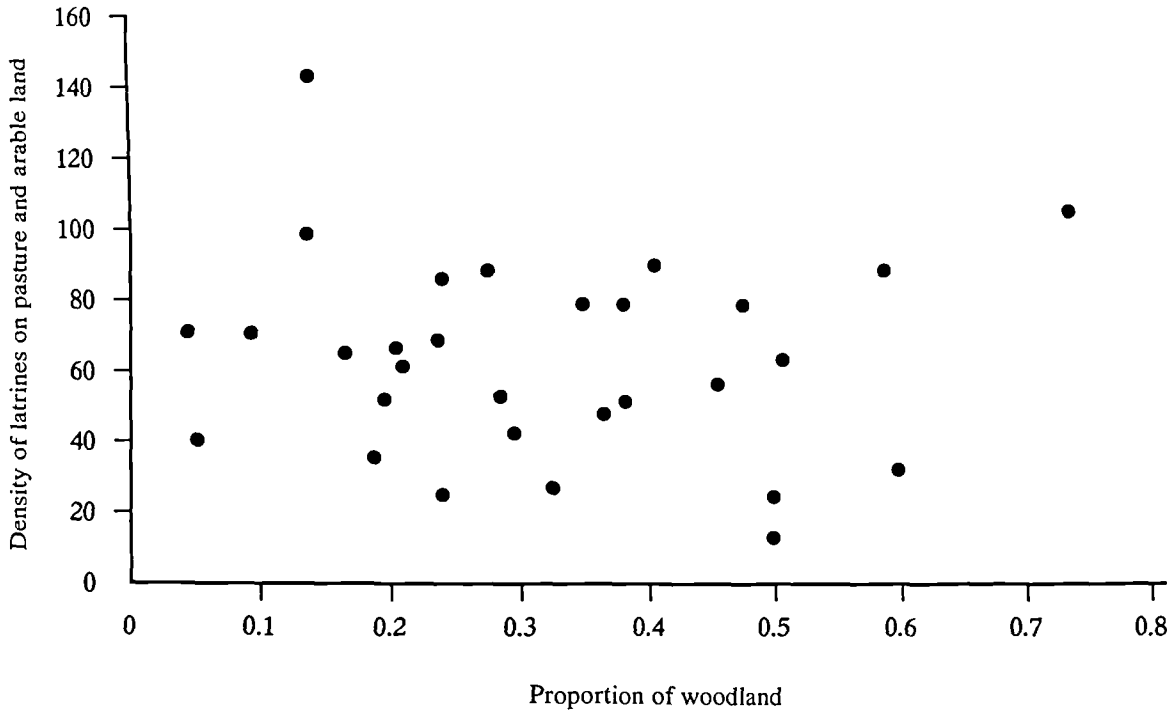


Figure 6.12 Relationship between the density of latrines on pasture and arable land and the proportion of woodland within badger territories ($F=0.14$, $R^2=0$, d.f.$=1,28$, $p>0.05$).



was found to be negatively correlated with territory size. For the Gloucestershire site, although not significant a trend was detected between latrine density and territory size ($F=5.94$, $R^2=49.7$, d.f.$=1,4$, $p=0.07$). No relationship between these variables existed for the area in Staffordshire ($F=0.69$, $R^2=0$, d.f.$=1,5$, $p>0.05$). No relationship between latrine density and group size was found for any of these sites.

6.3.6 Development of the predictive model

Stepwise multiple regression of overall latrine density on the five variables, proportion woodland (PW), proportion pasture and arable land (PPA), territory size (TS), group size (GS) and field boundary density (FBD), gave the best model (at 5% significance) with just two variables (TS and GS), although only explained 34.9% of the variation in latrine density (LD) ($F=8.79$, $R^2=34.9$, d.f.$=2,27$, $p=0.001$). The model took the following form:

$$LD = 81.81 - 126.16(TS) + 4.554(GS)$$

No transformation of any of the variables was found to improve this correlation.

The best model for explaining the observed variation in the density of latrines on pasture and arable land (DLPA) also included group size and territory size ($F=14.65$, $R^2=48.5$, d.f.$=2,27$, $p<0.0001$). Although this model was highly significant, the proportion of variation explained was rather low. This model was also found to underestimate pasture and arable latrine densities for relatively large territories. This was due to an exponential relationship between latrine density and territory size, and after a logarithmic transformation of all variables the predictive accuracy and the proportion of explained variation were improved

($F=23.75$, $R^2=61.1$, d.f.$=2,27$, $p<0.0001$), with the model taking the following form:

$$\text{Log}_e \text{ DLPA} = 1.7909 - 1.0608(\log_e \text{TS}) + 0.4650(\log_e \text{GS})$$

The final model estimated the number of latrines on pasture and arable land per km^2 of land (NLPA). Obviously this was dependent on the area of pasture and arable within a km^2, although the proportion of woodland within a km^2 was selected by the stepwise multiple regression as a factor of greater significance. Logarithmic transformation of the dependent variable increased the proportion of explained variation from 59.8% to 68.8% ($F=22.27$, $R^2=68.8$, d.f.$=3,26$, $p<0.0001$). The model took the form:

$$\text{Log}_e \text{ NLPA} = 4.6721 - 2.0123(\text{PW}) - 3.6332(\text{TS}) + 0.08075(\text{GS})$$

6.3.7 Testing the accuracy of the models

The accuracy of the models was tested with data supplied by the Ministry of Agriculture, Fisheries and Food for badger control operations conducted at sites in Cornwall, Avon, Gloucestershire and Staffordshire. The calculated latrine densities and predicted values for each social group at these four sites are presented in Table 6.1. In order that the reliability of the model could have been quantified when applied predictively to other areas, it would have been useful to have *a priori* criteria by which success could have been measured. The conventional statistical test of the correlation coefficient is inappropriate, since with a large volume of data a significant correlation can be achieved with a relatively small proportion of the total variation explained and with serious inaccuracies in prediction. Harris & Rayner (1986) assessed the performance of a model predicting fox density

Table 6.1 Surveyed and predicted values for the density of latrines on pasture and arable land and the number of latrines on pasture and arable land km^{-2} of territory, for the areas in Cornwall, Gloucestershire, Avon and Staffordshire; residual values are surveyed minus predicted values.

Surveyed density of latrines on pasture and arable land	Predicted latrine density	Residual value	Surveyed no. of latrines on pasture and arable land km^{-2} of territory	Predicted no. of latrines	Residual value
CORNWALL					
10.17	4.77	5.40	9.27	8.10	1.17
11.58	8.98	2.60	9.72	12.00	-2.28
11.18	17.41	-6.23	8.51	16.20	-7.69
21.20	15.77	5.43	14.68	13.87	0.81
21.47	26.32	-4.85	16.59	77.95	-61.36
7.62	6.19	1.43	6.46	8.00	-1.54
GLOUCESTERSHIRE					
23.49	22.84	0.65	18.23	43.35	-25.12
62.73	47.02	15.71	47.62	40.17	7.45
31.83	31.77	0.06	26.09	82.74	-56.65
25.64	24.67	0.97	13.84	23.04	-9.20
8.47	14.77	-6.30	1.73	6.44	-4.71
74.19	61.42	12.77	37.58	82.30	-44.72
AVON					
19.46	13.72	5.74	17.84	21.68	-3.84
23.81	22.92	0.89	23.60	65.02	-41.42
51.47	26.06	25.41	43.21	32.98	10.23
12.99	9.34	3.65	10.09	12.18	-2.09
54.35	38.28	16.07	42.37	27.84	14.53
13.46	13.65	-0.19	12.38	16.99	-4.61
11.08	21.20	-10.12	9.15	21.70	-12.55
STAFFORDSHIRE					
23.28	11.64	11.64	17.14	8.55	8.59
51.93	7.94	43.99	39.54	11 69	27.85
101.12	32.33	68.79	66.58	29.81	36.77
36.58	10.95	25.63	18.41	6.89	11.52
44.22	41.40	2.82	31.41	74.81	-43.40
15.93	24.56	-8.63	10.47	14.85	-4.38
18.05	25.61	-7.56	8.96	14.46	-5.50

empirically, with one of their criteria being that at least 50% of predicted values for squares must be within 0.5 family groups km^{-2}, and 75% within 1.0. In view of the difficulty in locating all latrines within a surveyed area, and with data collection performed by different people, it was unrealistic to expect a complete fit between estimated and surveyed latrine densities, although the model could not be useful if estimates were consistently poor.

For the predictions of latrine density on pasture and arable land, residual values of less than 5.0 occurred for 38.5% of social groups, 61.5% of predicted values were within 8.0 of surveyed latrine densities and predicted values within 20% of surveyed values occurred for 30.8% of the social groups. For the number of latrines on pasture and arable land per km^2, 42.3% of predicted values were within 6.0, 61.5% were within 9.0 of surveyed values, and predicted values occurred within 20% of surveyed values for 11.5% of the social groups.

6.4 Discussion

This analysis examined those factors that were important in regulating latrine density, and then used those particular parameters to develop models to predict latrine density outside the Cotswold escarpment study area. It was originally intended to base the analyses on map-derived habitat variables since this would have formed a simple and practical method of estimating latrine density and examining the potential for disease transmission. White, Brown & Harris (1993) have shown that for certain landscape types, habitat features were correlated with herds with reactor cattle and correctly classified with about 70% accuracy, random versus control squares simply on the basis of map-derived habitat variables. These authors hypothesised that the type and density of linear features, which influenced badger urinatory behaviour were a significant causative factor in the transmission of bovine tuberculosis to cattle.

Although the density of linear habitat features was found to be correlated with the density of latrines on pasture and arable land, other factors were shown to affect latrine density to a greater extent. Characteristics of the habitat such as field boundary density were not important factors in determining latrine numbers and this particular variable was dropped from the analyses during the stepwise multiple regression. This may indicate that the findings of White, Brown & Harris (1993) that high risk areas were characterised by greater field boundary density and habitat heterogeneity may be explained in terms of badger urinatory behaviour on crossing point runs and not because these areas were characterised by high latrine densities. Although, as latrine density on pasture and arable land was positively correlated with field boundary density, areas with high densities of field boundaries are likely to have higher densities of latrines on pasture with all other things being equal. One of the factors that could not be included in these analyses which was possibly of major significance was the type of field boundary in question. Chapter 5 showed that the type of field boundary was a key factor in determining latrine sites, with badgers creating latrines preferentially at those field boundary types with restricted access. The lack of data on field boundary type for the study area was probably the main reason why field boundary density was not a significant factor in the model since certain types of field boundary appear to be particularly important in establishing latrine sites. This omission from the model may explain to some extent the poor predictive capability of the model within particular areas. White, Brown & Harris (1993) found that habitat heterogeneity and field boundary density were only significant factors in TB transmission to cattle within certain types of landscapes. These landscapes may be characterised by having field boundary types that restrict badger movement, providing increased potential for urination and/or defecation. Analyses of the data for Staffordshire, Cornwall, Avon and Gloucestershire revealed that in only Cornwall and Avon field boundary density was significantly correlated with latrine density on pasture and arable land. It is possible that the

Avon and Cornwall sites were characterised by key field boundary types which are selected by badgers for urination and defecation. This would explain the relationship between field boundary density and latrine density for these areas. Also, White, Brown & Harris (1993) have shown that for the type of landscape found at the site in Avon, field boundary density was related to areas with repeated herd breakdowns in the south-west of England. Thus for these areas, where field boundary density is high, the density of latrines on pasture is also likely to be high which may be responsible for the repeated herd breakdowns in these areas. Clearly a survey to classify field boundary types in different regions is needed to improve the model and would be particularly important in evaluating the potential risk from crossing point urinations and latrines in different areas.

The major problem experienced in developing a model for estimating overall latrine density was the difference between boundary and hinterland latrines in their relationship with territory size and group size. Since the number of boundary latrines per social group varied little on the Cotswold escarpment, across a range of territory sizes, this resulted in a highly significant negative correlation between boundary latrine density and territory size. In fact this single parameter accounted for 40.8% of the variation seen in boundary latrine density and 47.1% after the logarithmic transformation of boundary latrine density. The positive relationship between the number of hinterland latrines on a territory and territory size, resulted in no relationship between hinterland latrine density and territory size.

Possibly of major significance in the transmission of TB to cattle was the finding that larger territories contained a greater number of hinterland latrines per badger. Using the linear regression equation for the regression line from Figure 6.7, 1.74 hinterland latrines per badger occurred on a territory of 0.279 km^2, the average territory size on the Cotswolds study site for 1991 and 1992 (C.L. Cheeseman unpublished). This figure rose to 4.17 for a territory size of 0.70 km^2, a common territory size in many areas of the south-west (Cheeseman *et al.* 1981).

This suggests that given a finite production of excreta by badgers, hinterland latrines on larger territories are likely to contain smaller quantities of excreta. This may have important implications in the transmission of bovine tuberculosis at lower badger densities, as latrines containing relatively small quantities of excreta may be investigated by cattle with greater frequency than larger latrines heavily contaminated with faeces.

For those species which mark throughout their range or territory it has been argued that the function of the deposited scent mark is to help orientate the individual producing the mark, maintaining the individuals familiarity with its range (Lyall-Watson 1964; Ralls 1971; Ewer 1973). This may explain why badgers living in large territories have a greater number of hinterland latrines per individual than for badgers on smaller territories. Alternatively if hinterland latrines are considered to have a defensive role against animals from neighbouring social groups, then the observed increase in hinterland latrine numbers with increased territory size may merely reflect the requirements of a social group defending a larger territory.

The relatively constant number of boundary latrines irrespective of group and territory size may indicate that only a small proportion of the social group is responsible for maintaining boundary latrines (i.e. dominant boar(s) and sow(s)). This situation occurs in the wolf, where the alpha male and female are almost exclusively responsible for the pack's scent marking (Peters & Mech 1975; Haber 1977). Yet Chapter 3 found that although adult males were the main instigators of territoriality in the form of boundary path patrolling and boundary scent marking, the division of labour in scent marking in the badger was not as well developed as that in wolves, with all members of the social group taking part to some extent in territorial behaviour. It is more probable that for badgers living in larger territories investing in hinterland latrines is a more profitable strategy than increasing boundary latrine numbers. Given a finite number of latrines, increasing latrine

numbers along the territory boundary would occur at the expense of hinterland latrines which, on a larger territory, would leave large areas without latrines.

Although boundary marking does provide the earliest warning to neighbouring social groups that a territory is occupied, it involves only a single line of defence which must be maintained intact and renewed regularly if overt conflicts are to be avoided. To maintain a dense, fresh line of scent marks along the territory boundary requires a large supply of scent relative to the length of perimeter to be marked. Given a limited time budget and a finite supply of excreta and scent secretion, as territory size increases it becomes progressively more difficult to visit and mark the border with the frequency and regularity necessary to maintain the continuity of the line of scent marks. Thus border marking is possible when the supply of scent is large compared to the length of perimeter to be marked.

Gorman & Mills (1984) believe that the length of boundary to be marked per individual group member is the critical parameter that determines if animals adopt the hinterland or boundary marking strategy. In a situation where a social group is faced with scent marking a long border, hinterland marking is perhaps a safer strategy, since although an intruder may cross the boundary and penetrate into the territory, sooner or later that individual will encounter a hinterland latrine. This is the case for brown hyaenas (*Hyaena brunnea*) in the Kalahari which live in small groups, the members of which share a large territory 235-480 km^2) (Mills 1976) and adopt the hinterland marking strategy (Mills 1982). This type of territory marking has also been recorded in the striped hyaena (*Hyaena vulgaris*), a solitary species which occupies a large territory (40-70 km^2) Kruuk 1976 . On the other hand the aardwolf (*Proteles cristatus*) which lives alone, or in pairs in the Serengeti, in a very small territory (1.5 km^2), adopts the boundary marking strategy (Kruuk & Sands 1972). Intra-specific variation in marking strategy has been recorded for the spotted hyaena. Spotted hyaenas living in the Kalahari live in relatively small groups and occupy large territories (c. 1000 km^2). The length of border per group

member is similar to that of the brown hyaena and here spotted hyaenas mark throughout their territory (Mills & Gorman 1987). Group sizes are larger for spotted hyaenas living in the Ngorongoro and territories considerably smaller than for Kalahari hyaenas (30 km^2). As a result each group member has only a relatively small length of perimeter to scent mark and here boundary marking is the adopted strategy. Figure 6.8 indicates that a similar phenomenon may be involved in the territorial behaviour of the badger. For those groups where the length of territory boundary to be marked per individual was relatively long, animals concentrated their scent marking behaviour throughout the territory. Although the number of hinterland latrines per individual rose with an increase in territory size, this did not explain the relationship seen in Figure 6.8, since the length of territory boundary per individual was not correlated with territory size. If the hinterland marking strategy is preferred when the length of boundary to be marked per individual is relatively large, then this strategy may be utilised by badgers living at low population densities. With a greater investment in hinterland marking, the deposition of urine on crossing point runs may occur more frequently at lower population densities. Data collected using spool-and-line and biomarker techniques at lower population densities in the south-west of England would be instrumental in testing this hypothesis. Neal (1986) has suggested that for badgers in large territories, boundary marking and patrolling along the whole of the perimeter becomes physically impossible and that dung pits become restricted to such places as good worming patches or where a seasonal abundance of food is found. Macdonald (1980) has also suggested that the distribution of latrines may reflect the distribution and quality of food patches. Perhaps an important omission from the model were parameters that adequately describe food availability and distribution.

In the present analyses, since it was intended to derive variables from map-derived habitat features, fields were not distinguished as permanent pasture or arable land. Although Chapter 5 showed that latrine densities were similar on

pasture (0.64 ha^{-1}) and arable land (0.70 ha^{-1}) these values were only based on five social groups and differences in the relative proportions of pasture and arable land between the Cotswold escarpment and the other four areas may have been a contributing factor in the inaccuracy of predicted latrine densities.

Apart from the different relationship between boundary and hinterland latrine densities with territory size, the other problem in modelling overall latrine density was that hinterland latrine density was significantly correlated with group size, while no such relationship existed for boundary latrines. The positive relationship between hinterland la rine density and group size may be borne out by a more complex social structure relying on a higher level of olfactory communication between group members. Alternatively the greater nightly output of excreta from a larger social group may explain this relationship. Since Chapter 4 found that fresh faeces appeared to stimulate defecation, defecations away from existing latrines are more likely to be encountered and marked by other badgers when group size is large. Given the higher latrine density for larger social groups, latrines are probably encountered with greater frequency by badgers living in larger social groups. Apart from the increased opportunities of tuberculosis transmission through direct contact between badgers living in larger groups, higher densities of latrines may act to accelerate tuberculosis transmission between group members, due to an increased probability of contacting infective excreta from infected individuals within the social group.

In conclusion, it was intended to analyse map-derived habitat variables which would have formed a practical method of estimating potential high risk areas. Although the model predicts latrine density reasonably accurately, knowledge of the types of field boundary on the four areas would probably greatly improve the predictive capability of the model. In its present state the model has few practical applications given the parameters included, although these analyses have revealed some interesting aspects of badger territoriality and the possible significance of this

behaviour in TB transmission. Although only preliminary, these analyses indicated that only within certain types of landscapes was field boundary density correlated with latrine density. This is consistent with White, Brown & Harris (1993) who found that field boundary density was only related to areas with repeated herd breakdowns within certain types of landscapes. Chapter 5 indicated a possible link between crossing point run urinations and latrines and it is hypothesised that types of landscapes in which field boundary density is related to repeated herd breakdowns are characterised by having high densities of field boundary types that restrict badger movement resulting in greater levels of contamination of pasture with badger urine and faeces and hence a greater chance of disease transmission. This highlights the need to survey field boundary types as this is probably one of the major factors that determines high risk areas in the south-west of England.

CHAPTER 7

GENERAL DISCUSSION

7.1 Tuberculosis transmission from badgers to cattle

The underlying objective of this thesis was to identify possible modes of transmission of bovine tuberculosis from badgers to cattle. Since cattle are believed to become infected while grazing pasture contaminated by badger excreta, this study concentrated on the excretory behaviour of badgers, identifying sites of excreta deposition and analysing seasonal patterns of dunging and urinating behaviour.

Probably one of the most significant findings in relation to TB transmission was the urinatory behaviour of badgers on crossing point runs through field boundaries. This thesis concentrated on these urinations as being potentially the major factor involved in TB transmission and may perhaps have over-emphasized the importance of these urinations, since at this stage the role of these urinations in the transmission of bovine tuberculosis from badgers to cattle is still largely speculative. However, the peak in the nightly excretion of urine on pasture crossing point runs occurred in the spring, the time of year that cattle are at greatest risk of infection. Wilesmith *et al.* (1982) have pointed out that cattle are at greatest risk of infection in April and May and believe that re-exposure to *M. bovis* occurred at this time each year over a relatively short period of time. The peak in contamination of pasture with badger urine occurred during the summer and for faeces in the autumn. Clearly these two peaks do not coincide with the high risk period for cattle, which may be more easily explained by the urinatory behaviour of badgers on crossing point runs.

The length of time that excreted bacilli remain infective on pasture is an important factor in the transmission of infection from badgers to cattle. In summer, *M. bovis* in badger urine and sputum have been reported to remain viable for only a few days (Ministry of Agriculture, Fisheries and Food 1979). This may explain why Wilesmith *et al.* (1982) found that cattle were only exposed to infection for a relatively short period of time (April and May). Although the number of crossing point urinations per badger per night on pasture (Table 4.2) continued at a relatively high level between March and August, it is possible that since bacilli remain infective for only a short period in the summer, infected urine deposited on pasture crossing points later than May poses little risk to cattle. Likewise the peak in urinations on pasture during the summer may be of reduced significance due to the poor survival of bacilli at this time. The rapid decomposition of faeces recorded during the summer is also likely to accelerate the destruction of *M. bovis*. Although bacilli in badger urine and sputum can remain viable for up to 10 weeks in winter (Ministry of Agriculture, Fisheries and Food 1979), the low level of pasture contamination with both urine and faeces during the winter months is consistent with the observations of Wilesmith *et al.* (1982), who report that cattle are at little or no risk of becoming infected during the winter.

The urinatory behaviour of badgers at crossing point runs was influenced largely by the type of field boundary, with badgers urinating preferentially at linear features which restrict badger movement to a limited number of crossing points. The positive correlation between the number of crossing point urinations and the number of field boundaries crossed suggests that if a similar relationship occurs in other problem areas in the south-west, the density of linear features may have an influence on the number of crossing point urinations. Since White, Brown & Harris (1993) have shown that field boundary density is correlated with herds with reactor cattle in particular types of landscapes in the south-west of England, this urinatory behaviour may be the significant causative factor behind this correlation. However,

the types of field boundary preferentially selected by badgers for urinating were also selected preferentially for establishing latrine sites and since in some parts of the south-west latrine density was related to field boundary density, the areas with repeated herd breakdowns in the south-west that contain high densities of field boundaries (White, Brown & Harris 1993) may be characterised by high latrine densities. Thus in these areas the influence of field boundaries on both urinatory and dunging behaviour would result in high levels of pasture contamination and therefore increased contact probabilities between infected excreta and cattle.

Although cattle would have been excluded from over 90% of these crossing point urinations if cattle were denied access to an area four metres from a field boundary, this represents a relatively large proportion of the field area, particularly in high risk areas with large field boundary densities and small fields. Since badgers select linear features with restricted access, perhaps a more practical recommendation would be to open up certain field boundaries to provide easier access to badgers. This may have the effect of reducing the significance of a field boundary as a site for urine marking and subsequently reduce the number of crossing point urinations on pasture. Alternatively badgers could be allowed access through just a small part of a boundary, with that area fenced from cattle.

7.2 Future work

At this stage these suggestions are not intended as recommendations, since the effect of manipulating field boundaries on badger excretory behaviour is unknown. Manipulations may have the effect of enhancing the problem and clearly further research is needed to examine how the level of contamination of pasture with badger urine may be reduced and/or how these urinations can be excluded from cattle. Given that faeces were deposited almost entirely at latrines and that at certain times of the year latrines received relatively large quantities of urine, the

exclusion of cattle from latrine sites and the effect of this on badger excretory behaviour also warrants investigation.

If badgers living at low densities adopt a hinterland marking strategy as has been suggested, this may involve a greater dependence on crossing point urinations. With this in mind a spool-and-line study on a low density population of badgers would be essential in testing this theory, as well as quantifying the potential risk to cattle from badger scent marking for a low density population. Given the significance of certain field boundaries in regulating the urinatory and dunging behaviour of badgers and since White, Brown & Harris (1993) have shown that field boundary density is correlated with herds with reactor cattle, more detailed information is required on the nature of field boundaries in different areas in the south-west of England and on the pattern of crossing point urinations in different areas.

Although cattle generally show strong avoidance of badger excreta, some cows have been shown to graze latrine areas and consume contaminated pasture when presented with turfs (Benham & Broom 1991). In the present study cattle were observed to eat pasture contaminated by badger urine on crossing point runs within 24 hours of its deposition. More information is needed on the contact probabilities with infected excreta by cattle and the avoidance and/or investigation of these products by cows, especially crossing point urinations. In particular research is needed to examine the longevity of survival of *M. bovis* on pasture since this can be incorporated into models estimating contact probabilities between cattle and infective excreta. Despite observations of cattle consuming and/or investigating contaminated pasture, the significance of these behaviours is unknown without quantified data on the seasonal pattern of *M. bovis* survival on pasture.

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APPENDIX

PUBLISHED WORK

1992 Studies on the spread of bovine tuberculosis from badgers to cattle. *Journal of Zoology, London*, 227, 694-696. (with C.L. Cheeseman & S. Harris)

1993 The development of field techniques for studying potential modes of transmission of bovine tuberculosis from badgers to cattle. *The badger* (ed. T.J. Hayden). Royal Irish Academy, Dublin. In press. (with S. Harris & C.L. Cheeseman)

1993 Badgers (*Meles meles*), cattle and bovine tuberculosis (*Mycobacterium bovis*): a hypothesis to explain the influence of habitat on the risk of disease transmission in south-west England. *Proceedings of the Royal Society of London, Series B*, 253, 277-284. (with P.C.L. White & S. Harris)

As the second listed publication is largely contained within this thesis only the abstract is reproduced here. The first and third listed publications are reproduced in full.

Studies on the spread of bovine tuberculosis from badgers to cattle

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Bovine tuberculosis in cattle continues to be a problem in Britain, particularly in south-west England, where the number of herd breakdowns in 1990 was 104, compared with only 39 herd breakdowns in the whole of the rest of Britain (MAFF, 1991). Evidence linking badgers with the spread of bovine tuberculosis in the south-west of England has accumulated since the discovery of the disease in a badger in Gloucestershire in 1971 (Muirhead, Gallagher & Burn, 1974; Gallagher, Muirhead & Burn, 1976; MAFF, 1976, 1977, 1979).

The route of transmission of bovine tuberculosis from badgers to cattle has been little studied and remains speculative. It is believed that cattle may pick up infection while grazing pasture contaminated by an infected badger (Muirhead *et al.*, 1974; MAFF, 1979), and that contamination of pasture and cattle food could result from urine, faeces and/or sputum from an infected badger (Muirhead *et al.*, 1974). In addition, infected badgers inhabiting farm buildings may give rise to 'high risk' situations, especially if cattle food is contaminated (Cheeseman & Mallinson, 1980).

Contaminated badger urine would appear to pose a greater risk in the process of transmission than faeces (Brown, Harris & Cheeseman, 1992). However, there are no quantified data on the pattern and frequency of urine distribution on badger territories, nor any information on the effects of season, sex or social status on the pattern of urination. Considerably more is known about the overall deposition of faeces on territories, but little is known about the influence of social factors on a badger's dunging behaviour. Badgers defecate in small open pits, and at certain places within the territory these are aggregated to form latrines which serve for territorial defence (Neal,

1977; Kruuk, 1978). A second type of defecation site, termed a temporary defecation site, has been identified, but these probably are not concerned with territorial defence, and are used only once or twice before being abandoned (Roper, Shepherdson & Davies, 1986). Badgers also deposit anal gland secretion at latrines either on top of faeces or in separate pits.

With an improved understanding of the factors affecting the deposition of faeces and urine by badgers on their territories, it should be possible to identify ways of reducing contact between cattle and badger excretory products, and hence ultimately reduce the spread of bovine tuberculosis from badgers to cattle. That is the ultimate aim of this study. However, to understand the pattern of urine and faeces deposition by badgers, it was first necessary to develop new study techniques.

Selected animals were caught in cage traps, fitted with a spool-and-line, and injected subcutaneously with fluorescein dye; this biomarker was subsequently excreted in the urine, faeces and anal gland secretion (Brown *et al.*, 1992). Traps were set in the middle of the night, so that badgers were caught returning to the sett after foraging. This was to ensure that the period in captivity was as brief as possible, and that food intake, and hence excretory behaviour the following night, were not affected. Traps were checked at first light, and animals released within one hour of anaesthesia at the entrance hole closest to the point of capture. The following night the line was followed from the time the animal emerged until it finally returned to a sett; the tracker was, on average, about 30 min behind the badger. In addition, radio fixes were recorded at 15 min intervals to provide a time frame for the spool-and-line data. A lamp emitting ultra-violet light of 366 nm wavelength was used to identify the fluorescein labelled urine, faeces and anal gland secretions deposited by the study animal.

Whilst a full year's data are not yet available, the preliminary results show for the first time the potential importance of urine as a scent marker in territorial behaviour, particularly in adult males. Adult male badgers urinated significantly more at boundary latrines than adult females ($\chi^2 = 6{\cdot}30$, 1 *d.f.*, $P < 0{\cdot}05$), and also visited significantly more boundary latrines than adult females ($\chi^2 = 10{\cdot}48$, 1 *d.f.*, $P < 0{\cdot}01$). Analysis of variance showed that adult males urinated significantly more times per night than all other age and sex classes (Tukey's HSD, $F = 7{\cdot}09$, 5 *d.f.*, $P < 0{\cdot}001$); however, there was no significant difference in the mean number of faeces produced per night (Tukey's HSD, $F = 0{\cdot}65$, 5 *d.f.*, $P > 0{\cdot}05$). During the summer (June–August) there was a marked lack of excreta from cubs. In this period a total of eight different cubs were spool-and-line tracked throughout the night, but only one of these animals defecated, once at a hinterland latrine, and none produced any urine. Because so little excreta from cubs was found above ground, they were probably eliminating urine and faeces below ground. Other age classes also showed a low production of faeces above ground at certain times of the year. Latrines, sometimes containing large numbers of fresh faeces, have been found below ground in a number of excavated setts, and these were in empty chambers as well as in tunnels (S. Harris and C. Cheeseman, unpubl.; Roper *et al.*, 1991). Hence it is possible that badgers defecate below ground considerably more frequently than previously believed; the extent to which this occurs is currently being investigated. Cubs also urinated and defecated infrequently in the autumn (September–November), and of particular interest was that they appeared to urinate randomly on pasture. This phenomenon was rarely observed in adults and yearlings, where 97·3% of all their urinations on pasture occurred at latrines or in association with badger runs.

With adults and yearlings depositing such a high proportion of their urine, and all of their faeces, at strategic sites on pasture, it should be possible to reduce greatly the contact between badger excreta and cattle, especially if cattle are denied access to latrines. Further work will

investigate the effects on badger excretory behaviour of manipulating badger latrines by the addition or removal of faeces and urine, as well as the effects of moving or removing entire latrines. Monitoring the response of individuals of known age and sex to faeces and urine from known individuals will improve our understanding of the way in which these products are used for communication between badgers, both within and between social groups. Furthermore, it may be possible to prevent badgers from contaminating pasture by identifying the reasons why certain sites are used for latrines by badgers, in areas where TB is a regular problem. This should help to reduce the incidence of TB in cattle.

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The development of field techniques for studying potential modes of transmission of bovine tuberculosis from badgers to cattle

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Abstract

The routes of transmission of tuberculosis from badgers to cattle are still largely speculative, and so novel techniques have been developed to help understand these processes. Spool-and-line tracking in conjunction with a biomarker were used to locate sites of badger urination and defecation. A 0.38 g ml^{-1} solution of fluorescein LT injected subcutaneously was found to inhibit *Mycobacterium bovis*, while large numbers of bacilli were recovered with a 0.22 g ml^{-1} solution. Thus urine from infected badgers injected with a 0.22 g ml^{-1} solution of fluorescein LT was located at night using a portable ultra-violet lamp, so that contaminated grass samples could be removed for subsequent bacterial culture to establish the longevity and vertical distribution of *M. bovis* on pasture. Behavioural interactions between badgers and cattle, and of cattle to contaminated pasture, were also recorded. These techniques will help identify the possible routes of transmission of bovine tuberculosis from badgers to cattle.

Badgers (*Meles meles*), cattle and bovine tuberculosis (*Mycobacterium bovis*): a hypothesis to explain the influence of habitat on the risk of disease transmission in southwest England

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SUMMARY

Badgers are believed to be responsible for a high proportion of the cases of bovine tuberculosis in cattle in southwest England where, despite the onset of badger control operations in 1975, comparatively high numbers of cattle continue to fail the tuberculin test. To determine why the disease remains a problem in these areas, data on badger densities and patterns of land use were examined. Areas subject to repeated badger control operations had greater landscape heterogeneity and a higher density of linear habitat features. These habitat features were not related to badger density, measured as the mean number of social groups per square kilometre. Environmental contamination by infected badger urine is thought to be the main mode for the transmission of bovine tuberculosis. Field studies in an area with tuberculosis in both badgers and cattle showed that badgers may urinate on pasture after crossing through a linear feature, and that the number of these crossing-point urinations increases with the number of linear features crossed. The hypothesis is presented that these crossing-point urinations are a major source of bovine tuberculosis infection in cattle, and that areas with increased numbers of linear features have greater levels of contamination of pasture with badger urine and hence increased opportunities for disease transmission.

1. INTRODUCTION

Since the discovery of a tuberculous badger (*Meles meles*) in Gloucestershire in 1971 (Muirhead *et al.* 1974), badgers have been thought to be responsible for a high proportion of the cases of bovine tuberculosis (*Mycobacterium bovis*) in cattle in southwest England (Zuckerman 1980). Yet little is known about how bovine tuberculosis might be transmitted from badgers to cattle. Seriously infected badgers may show aberrant behaviour patterns that bring them into close contact with housed cattle or their feedstuffs (Cheeseman & Mallinson 1981). However, such seriously infected animals are now rare in southwest England, and it is presumed that the majority of cases in cattle are due to contact with grass contaminated by badger urine, faeces or sputum (Muirhead *et al.* 1974). Of these, urine is thought to pose the greatest risk, because it contains large numbers of bacilli (up to 300000 per millilitre) (Gallagher & Horwill 1977).

To try to reduce the incidence of tuberculosis in cattle, badger control operations have been in force in southwest England since 1975. When one or more animals in a cattle herd react positively to the tuberculin skin test (the terms reactor cattle and herd breakdown are used hereafter), there is a detailed investigation of all the possible origins of infection by the Ministry of Agriculture, Fisheries and Food (MAFF); when badgers are considered to be the most likely source, a badger control operation is usually initiated.

Gassing setts with hydrogen cyanide was the main control method from August 1975 until June 1982. From August 1982, cage trapping replaced gassing. In this strategy, badger social groups were removed in a centrifugal manner until a 'ring' of uninfected social groups had been removed; mean size of the resulting control areas was 7 km^2, the same as for the gassing campaign (J. W. Wilesmith, personal communication). From April 1986 an 'interim' strategy was introduced, whereby badger trapping operations were confined to land used by the reactor cattle, or the whole farm if it was not possible to identify where the cattle were likely to have become infected. This strategy continued until the end of the work described here. Despite these various control operations, there has been no significant decline in the number of herds in the southwest with reactor cattle (MAFF 1993), and bovine tuberculosis in cattle is still largely confined to the same limited areas (about 12% of the total land area) in southwest England.

This paper examines whether specific habitat features may be significant in identifying these problem areas through their influence on badger excretory behaviour and hence the risk of transmission.

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Table 1. *Descriptions of the typical topography and land use found in land classes 1, 2, 5, 6, 7 and 17 (adapted from Bunce* et al. *1981b)*

(The availability of each land class is given as the percentage of the total land area in the southwest. Figures for mean badger density (groups per km²), percentage arable land and percentage pasture are from the 1 km squares surveyed in southwest England as part of the national badger survey (Cresswell *et al.* 1990); means are given ± s.e. Numbers of squares sampled: land class 1, 71; land class 2, 84; land class 5, 27; land class 6, 59; land class 7, 26; land class 17, 11.)

land class	description
1	Flat or gently undulating, little surface drainage, medium to low altitude, varied landscapes with trees and hedges, cereals and good grasslands. Proportion of land area, 20.5%; mean badger density, 0.63 ± 0.12 groups per km²; arable land, 32.1 ± 3.4%; pasture, 45.0 ± 3.3%.
2	Low ridges with sweeping curves, medium to low altitude, mainly open with few hedges, good grassland but extensive cereals. Proportion of land area, 18.0%; mean badger density, 0.48 ± 0.08 groups per km²; arable land, 45.7 ± 3.6%; pasture, 29.6 ± 3.1%.
5	Uniform gentle slopes, mostly at low altitude, many natural features, mixed farmland but predominantly good grass. Proportion of land area, 10.6%; mean badger density, 0.67 ± 0.15 groups per km²; arable land, 25.1 ± 4.9%; pasture, 47.6 ± 5.4%.
6	Dissected plateaux with many small rivers, complex topography with broad even slopes, medium to low altitude, many small fields enclosed by hedges, small woodlands, mainly good grassland with some barley. Proportion of land area, 33.2%; mean badger density, 0.54 ± 0.12 groups per km²; arable land, 21.2 ± 2.9%; pasture, 56.1 ± 3.0%.
7	Variable coastal morphology, mainly cliffs cut into plateaux, mostly low altitude, mainly good grassland with some arable. Proportion of land area, 4.1%; mean badger density, 0.37 ± 0.17 groups per km²; arable land, 19.7 ± 4.1%; pasture, 25.9 ± 3.9%.
17	Marginal uplands, plateaux often dissected by small rivers, medium to high altitude, mainly quite steep hillsides, mainly pastures with some good grasslands. Proportion of land area, 5.2%; mean badger density, 0.18 ± 0.13 groups per km²; arable land, 4.6 ± 4.9%; pasture, 62.6 ± 7.2%.

2. METHODS

(*a*) *Badger control operations*

For those counties in southwest England in which the majority of badger control operations were undertaken (namely Avon, Cornwall, Devon, Gloucestershire, Somerset and Wiltshire), 1 km squares where badger control had taken place between August 1975 and December 1991 were identified by using the MAFF's database. As badger control was only initiated following a herd breakdown that was attributed to badgers, the 1 km squares used in all subsequent analyses were predominantly those in which one or more herds had had reactor cattle which were thought to be due to infection transmitted from badgers.

The Institute of Terrestrial Ecology's land classification system (Bunce *et al.* 1981*a*, *b*) was used as a framework to examine the land use characteristics of squares subjected to badger control operations. This classification assigns every 1 km square in Britain to one of 32 land classes with similar climatological and topographical characteristics, and therefore provides a basis on which to stratify an analysis involving habitat variables over a large geographical area. For land classes with more than 100 control operations in total, the following variables were measured from 1:25000 maps, for samples of random and control operation squares: length of field field boundaries (metres), length of field road boundaries (metres), length of field deciduous woodland boundaries (metres), length of field coniferous woodland boundaries (metres), length of field mixed woodland boundaries (metres), area of deciduous woodland (hectares), area of coniferous woodland (hectares), and area of mixed woodland (hectares). Lengths of linear features were calculated from the index described in Olson (1950), using a grid cell size of 250 m or 125 m. Areas were measured to the nearest hectare by overlaying a 1 ha grid. The following additional variables were calculated from the map-derived ones: total field boundary length (metres), length of all field woodland boundaries (metres), total woodland area (hectares), heterogeneity of deciduous woodland, heterogeneity of coniferous woodland, heterogeneity of mixed woodland, and total woodland heterogeneity. Heterogeneity of a particular habitat type was calculated as follows: heterogeneity $= (l+1)/1000a$, where l is the boundary length of the feature in metres, and a is its area in hectares. Thus more fragmented habitats, i.e. those comprising a larger number of smaller patches, would have a greater total boundary length to area ratio, and hence a higher heterogeneity index, than more uniform ones. The Simpson's diversity (Simpson 1949) of land classes in the eight squares immediately surrounding a focal one was also calculated.

Analysis of variance (ANOVA) was used to compare individual map-derived habitat variables from squares subject to control operations with those from randomly selected squares within the same land class. For analyses on repeat (two or more) control operation squares, 50 random and, where possible, 50 squares subject to repeated control, were used from each land class; for single control operation squares, 25 random and 25 squares subject to control were used. Although one of the assumptions of ANOVA is that the samples being compared should have equal variances, the test is robust and operates well even with considerable heterogeneity of variances, provided that the sample sizes are similar (Zar 1984). Only those variables having non-zero values in more than 20% of the squares considered for each land class were included in the analyses. Because this approach required separate comparisons of 16 different variables between random squares and those subject to repeat control operations

Table 2. *Univariate factors for which $p \leqslant 0.1$ in the comparisons between squares subject to two or more control operations and random squares for land classes 1, 2, 5, 6, 7 and 17*

(Asterisks denote variables significant at $p \leqslant 0.01$ (see text); 50 random squares were used for all land classes, 50 repeat control squares for land classes 1, 2 and 6, 49 for land class 5, 27 for land class 7, and 45 for land class 17. Means are presented ± s.e.)

land class	variable	means		F value	significance (p)
		random squares	control squares		
1	Neighbouring land class diversity*	1.43 ± 0.07	1.74 ± 0.09	7.57	< 0.01
	Heterogeneity of deciduous woodland	0.12 ± 0.02	0.22 ± 0.03	5.07	0.03
	Heterogeneity of total woodland	0.15 ± 0.03	0.24 ± 0.03	4.06	0.05
2	Field total woodland boundaries/m*	895 ± 142	1980 ± 229	8.27	< 0.01
	Total woodland area/ha*	6.05 ± 1.25	12.21 ± 1.77	8.27	< 0.01
	Field deciduous woodland boundaries/m*	451 ± 106	1116 ± 227	7.17	< 0.01
	Area of deciduous woodland/ha*	2.92 ± 0.90	7.91 ± 1.77	6.47	0.01
	Field mixed woodland boundaries/m	385 ± 93	781 ± 181	3.86	0.05
5	All field boundaries/m*	8885 ± 431	10924 ± 473	10.37	< 0.01
	Field field boundaries/m	5335 ± 339	6644 ± 425	5.94	0.02
	Field road boundaries/m	2453 ± 226	2948 ± 204	2.70	0.10
6	Neighbouring land class diversity	1.39 ± 0.07	1.20 ± 0.06	4.48	0.04
7	Neighbouring land class diversity*	1.87 ± 0.13	1.38 ± 0.13	6.35	0.01
	Heterogeneity of total woodland	0.16 ± 0.04	0.07 ± 0.02	3.41	0.07
	Field total woodland boundaries/m	666 ± 146	298 ± 122	2.90	0.09
	Field field boundaries/m	5415 ± 409	6612 ± 619	2.85	0.10
17	All field boundaries/m*	5629 ± 611	10085 ± 767	21.47	< 0.01
	Field field boundaries/m*	3581 ± 450	6649 ± 600	17.52	< 0.01
	Field road boundaries/m*	1275 ± 201	2444 ± 261	13.16	< 0.01
	Heterogeneity of deciduous woodland*	0.09 ± 0.02	0.19 ± 0.04	6.39	0.01
	Heterogeneity of total woodland	0.13 ± 0.03	0.22 ± 0.03	5.18	0.03

within each land class, Bonferroni's adjustment was used to determine an appropriate level of significance for pairwise comparisons. For 16 comparisons at a maximum overall error rate of $\alpha = 0.20$, the significance of pairwise comparisons (α') was set at 0.01 (Howell 1982). For large numbers of comparisons, such as here, it is necessary to set α at no less than 0.20, because otherwise α' becomes too small and all the differences are masked. However, it also follows that the resulting p-values should be used only as a guide rather than significance levels in their own right (see Discussion). Logistic regression was used to compare squares subject to repeat and single control operations separately with the randomly selected squares. Habitat variables showing univariate differences at $p \leqslant 0.1$ were used as potential predictor variables, and selected by using a stepwise procedure (Capen *et al.* 1986).

(*b*) *Badger density*

For those land classes with more than 100 control operations, badger density (social groups per square kilometre) and habitat data were obtained from all the 1 km squares surveyed in the same six counties during the national badger survey (Cresswell *et al.* 1990). Each of the habitat variables was expressed as a percentage of the total non-built-up land, adjusted for any area of sea, within each square surveyed. Additional variables included were indices of landscape heterogeneity and hilliness. Landscape heterogeneity was measured as the sum of the number of different land use types occurring in 30 100 m × 100 m squares within three 100 m wide north–south transects of the 1 km square, and hilliness as the sum of the number of 25 ft contour lines within the same 30 squares. Badger density was compared with these variables by land class using correlations. Only those habitat variables which were recorded in more than 20% of the squares surveyed in each land class were included in the analysis for that land class.

(*c*) *Badger excretory behaviour*

This was studied using five badger social groups living on the Cotswold escarpment in Gloucestershire, an area that consists predominantly of land classes 1 and 2, with bovine tuberculosis in both badgers and cattle (Wilesmith 1983). For the five study groups, all in land class 2, mean group size (± s.e.) was 17.0 ± 2.8 ha, and mean territory size 37.2 ± 7.1 ha (C. L. Cheeseman, unpublished results). Mean areas of the main habitat types within these five territories (measured from maps of scale 1:2500) were: pasture 25.4 ± 2.9 ha; woodland 6.2 ± 2.4 ha; arable 3.7 ± 2.9 ha. Mean length of linear features was 10705 ± 2896 m per square kilometre. Cage traps were set early in the morning so that animals were caught as they returned to their sett after a night's foraging. A spool-and-line was fitted to a radio collar already worn by the badger, the animal was given a subcutaneous injection of fluorescein dye, and returned to its sett within 2 h. The night following capture, the line was followed from the time the animal emerged until it finally returned to the sett, and an ultraviolet light of wavelength 366 nm was used to identify fluorescein-marked excreta deposited by the focal animal (Brown *et al.* 1992). This technique had no detectable effect on

Table 3. *Summary of the logistic regression analyses comparing squares subject to repeat badger control operations with random squares for land classes 1, 2, 5, 6, 7 and 17*

(50 random squares were used throughout, 50 repeat control squares for land classes 1, 2 and 6, 49 for land class 5, 27 for land class 7, and 45 for land class 17. Variables used in the models: land class 1, neighbouring land class diversity, heterogeneity of deciduous woodland; land class 2, field–total woodland boundaries; land class 5, all field boundaries; land class 6, neighbouring land class diversity; land class 7, neighbouring land class diversity, heterogeneity of total woodland; land class 17, all field boundaries. The model χ^2 is the difference between -2 log likelihood for the model with a constant only, and -2 log likelihood for the current model. It therefore tests the null hypothesis that the coefficients for all of the terms in the current model, except the constant, are zero (Norušis 1990).)

land class	n	model χ^2	d.f.	p	% correct classification
1	100	12.56	2	0.002	67
2	100	15.77	1	< 0.001	68
5	99	10.14	1	0.001	57
6	100	4.53	1	0.033	55
7	77	14.09	2	< 0.001	75
17	95	19.24	1	< 0.001	63

the behaviour of the focal animal (Brown *et al.* 1993). Seasons were defined as: spring, March–May; summer, June–August; autumn, September–November; and winter, December–February.

3. RESULTS

(a) *Badger control operations*

Of the 16 land classes in the southwest, only six (i.e. numbers 1, 2, 5, 6, 7 and 17) had more than 100 control operations, and were therefore used in these analyses; they formed 91.6% of the land area in the southwest, and are described in table 1. Land classes which form only a very small proportion of the total land area and have few control operations were excluded because they would have biased any statistical analysis through a relative increase in the influence of chance causative events. The univariate factors that were used in comparisons between the random squares and those having undergone two or more badger control operations for each of the land classes are shown in table 2. Although the same habitat features were not consistently significant across all land classes, repeat control operation squares were generally associ-

Table 4. *Significant correlation coefficients (r) between badger group density and the habitat variables collected during the national badger survey (Cresswell* et al. *1990)*

(Numbers of 1 km squares sampled: land class 1, 71; land class 2, 84; land class 5, 27; land class 6, 59; land class 7, 26; land class 17, 11.)

land class	habitat variable	r	p
1	None	—	
2	Length of treelines	−0.28	0.01
	Area of arable land	−0.30	< 0.01
	Hilliness	0.31	< 0.01
5	Hilliness	0.55	< 0.01
6	Area of broad-leaved plantations	0.40	< 0.01
	Area of tall scrub	0.27	0.04
	Area of natural running water	0.28	0.03
	Area of lowland unimproved grassland	0.34	0.01
	Hilliness	0.29	0.03
7	Area of low scrub	0.39	0.05
17	None	—	

Table 5. *Mean number ± s.e. of urinations, and mean proportion ± s.e. of total number of urinations per badger per night at crossing-point runs in all habitat types*

(Comparisons of males (adults and yearlings combined) with females (adults and yearlings combined) were made by using Kruskal–Wallis single factor analysis of variance by ranks (Zar 1984). Mean number of urinations: males 0.59 ± 0.29, females 0.90 ± 0.20, $H = 3.25$, d.f. = 1, $p > 0.05$. Mean proportion of urinations: males 0.14 ± 0.06, females 0.36 ± 0.07, $H = 4.57$, d.f. = 1, $p < 0.05$.)

	number of animals	number of nights	mean number	mean proportion
adult males	9	16	0.75 ± 0.49	0.13 ± 0.09
adult females	15	28	0.81 ± 0.24	0.31 ± 0.09
yearling males	7	13	0.36 ± 0.19	0.15 ± 0.08
yearling females	4	4	1.25 ± 0.29	0.54 ± 0.05

Table 6. *Mean number ± s.e. of urinations per badger per night on crossing-point runs by season; all age and sex classes combined*

	number of animals	number of nights	crossing-point runs on all habitat types	crossing-point runs on pasture only
spring	20	23	0.63 ± 0.17	0.53 ± 0.16
summer	17	20	0.71 ± 0.34	0.44 ± 0.25
autumn	17	21	0.12 ± 0.08	0.12 ± 0.08
winter	20	25	0.35 ± 0.19	0.23 ± 0.16

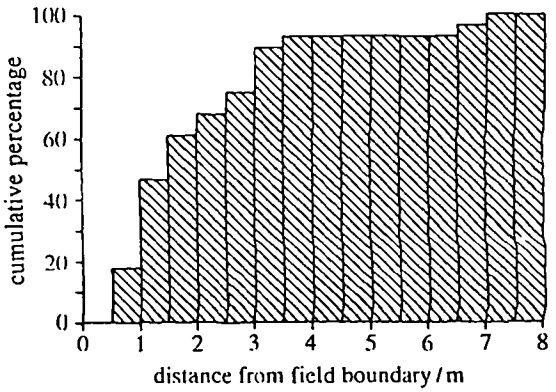

Figure 1. Cumulative percentage of distances of crossing-point urinations from field boundaries; the figure shows the furthest point of the trail or patch of urine from the field boundary.

ated with an increased density of linear features and greater habitat heterogeneity within the land classes examined. This pattern was consistent, but at a slightly lower level of significance, for single control operation squares. For land class 6, the diversity of land classes of neighbouring squares was lower for control compared with random squares, suggesting that homogeneous areas of land class 6 were more susceptible to cattle herd breakdowns.

Table 3 summarizes the results of the logistic regressions on repeat control operation against random squares for each of the six land classes. Logistic regressions distinguishing random from repeat control operation squares were significant ($p < 0.05$) for all the land classes considered, and correctly classified between 55% and 75% of all squares in the analysis.

(b) Badger density

For each of the six land classes examined, the significant ($p < 0.05$) correlation coefficients between badger density and the habitat variables collected during the national badger survey are shown in table 4. Badger density was positively correlated with hilliness in land classes 2, 5 and 6, and with scrub and/or woodland in land classes 6 and 7. However, badger density in southwest England was not correlated with landscape heterogeneity or the length of hedgerows in any of the land classes considered.

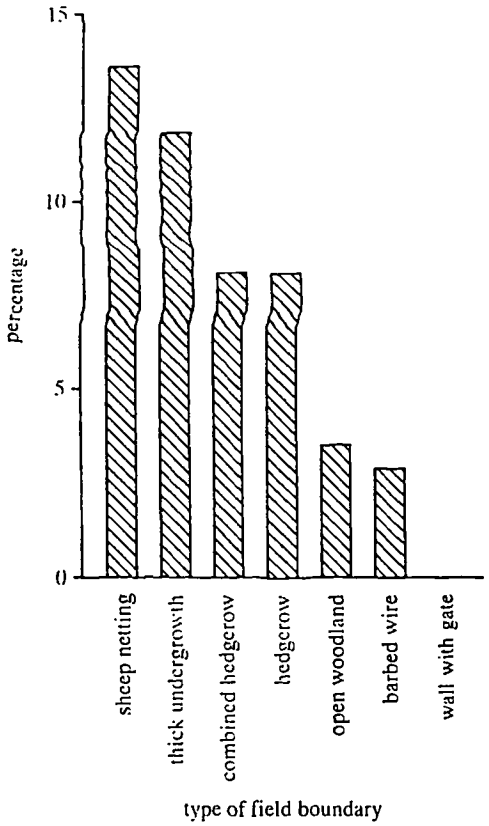

Figure 2. The number of urinations deposited by badgers at specific field boundary types, expressed as a percentage of the total number of times these boundaries were crossed. The number of urinations deposited and field boundaries crossed were summed across all badgers; where a badger was followed more than once, a mean value was used for that animal. Combined hedges are those in conjunction with either barbed wire, sheep netting or a wall.

(c) Badger urinatory behaviour

From June 1991 to May 1992, 93 complete nights' spool-and-line data were obtained from 42 different animals. Of the 125 urinations recorded, 24% were at latrines in woodland, 28% at latrines on pasture, and 28% on pasture away from setts and latrines; the remaining 20% were in woodland away from latrines or on setts. Of those on pasture away from setts and

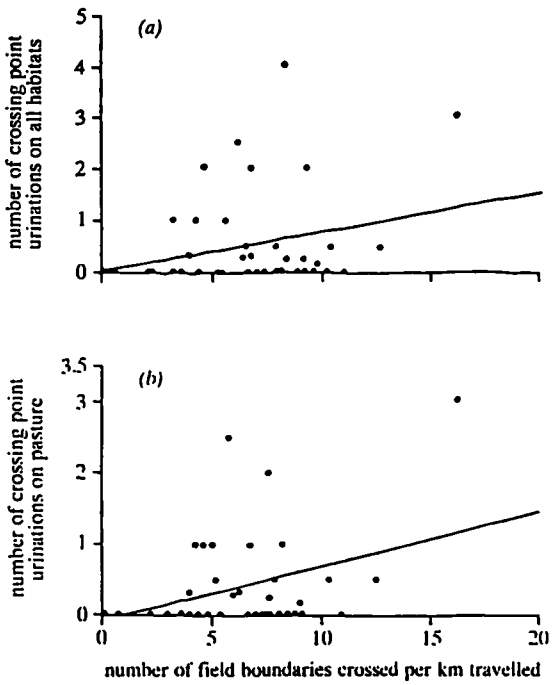

Figure 3. Relation between the number of crossing-point urinations and number of field boundaries crossed per kilometre travelled for (*a*) all habitat types ($r = 0.33$, d.f. = 1, 40, $p < 0.05$) and (*b*) pasture only ($r = 0.40$, d.f. = 1, 40, $p < 0.01$).

latrines, 3% were on boundary runs, 83% associated with runs crossing linear features (crossing-point urinations) and 14% were random (i.e. away from runs). Only adult sows and cubs urinated randomly. Cubs did not urinate at crossing points, and for the other age and sex categories both the mean number and mean proportion of urinations at crossing-points were highest for females (table 5). The seasonal distribution of urinations on crossing point runs is shown in table 6. Urinations on these runs either took the form of trails (mean proportion 0.61 ± 0.09) or patches (mean proportion 0.39 ± 0.09). At crossing-point runs on pasture, trails measured up to 1.60 m in length (mean 0.75 ± 0.12 m), with a mean patch diameter of 0.13 ± 0.01 m. They were normally deposited on the badger path just after the badger had passed through the linear feature, but occasionally when it was travelling parallel to the linear feature. Although over 90% of the crossing-point urinations on pasture were within 4 m of the linear feature, a small proportion were deposited up to 7.5 m away (figure 1).

The proportions of crossing-point urinations at different types of field boundary are shown in figure 2. Badgers urinated more than expected at crossing points on boundaries with restricted access (sheep netting, thick undergrowth and hedges), and less than expected at crossing points on boundaries with less restricted access (open woodland, barbed wire and walls with gates) ($\chi^2 = 6.61$, d.f. = 1, $p < 0.05$). The number of crossing-point urinations was significantly correlated with the number of boundaries crossed per badger per night per kilometre travelled both for all habitat types and for pasture alone (figure 3); thus there was an increase in the number of crossing-point urinations with the number of boundaries crossed. Taking account of the mean group size for the five study groups, and using the mean number of urinations for each age and sex category, the projected number of crossing-point urinations on pasture per night per group is 6.2 in spring, 7.7 in summer, 1.5 in autumn and 2.4 in winter. Including random urinations on pasture, these figures become 7.5, 7.7, 4.5 and 2.4, respectively.

4. DISCUSSION

This analysis examined habitat features in those areas of southwest England where badgers were believed to be the source of infection for reactor cattle. During the centrifugal trapping policy, some 1 km squares in which no reactor cattle had been recorded were also subject to badger control operations. However, the number was small and their inclusion in the analyses had no statistical effect on the overall results. The possibility that differences between random and control operation squares were due to differences in land use was minimized by confining all the present analyses to within land classes; each land class has its own characteristic patterns of land use (Bunce *et al.* 1981*b*).

Ever since badgers were first implicated as a cause of herd breakdowns in southwest England, the mode of transmission of bovine tuberculosis from badgers to cattle has remained largely speculative. Although environmental contamination with the excretory products of infectious badgers, especially urine, has long been suspected to be the most likely source of infection, the situations in which cattle make contact with (i.e. investigate and/or consume) these excretory products remains unknown. Benham & Broom (1991) showed that cattle very strongly avoided pasture contaminated with badger faeces, although their avoidance of badger urine was less pronounced. Also, it has generally been believed that most badger urine is deposited at latrines, and these are avoided by cattle because of the faecal contamination of the area. During our study, however, cattle were observed to eat pasture contaminated with urine at crossing-point runs within 24 h of deposition (J. Brown, unpublished results), suggesting that urine deposited away from latrines is less strongly avoided, and hence poses a significant risk of transmission.

Despite nearly two decades of badger control operations, the fact that the areas in southwest England that experience problems with herd breakdowns remain limited in area and largely unchanged suggests that these areas are characterized by one or more features associated with the disease transmission process. Caution should be used in trying to isolate specific key habitat features within a particular land class based on significant univariate differences between random and control operation squares, due to the multiple tests used and the non-independence of the habitat variables within a sample of squares from the same land class.

However, consistent associations between certain types of habitat feature (i.e. landscape heterogeneity and the density of linear features) and areas of repeated badger control within certain land classes, and the ability of logistic regressions to distinguish between random and control operation squares with up to 75% accuracy, based simply on map-derived variables, suggest that these types of habitat feature are associated with an enhanced risk of disease transmission in these land classes. We argue that this is because linear features which restrict badger movements to a limited number of crossing points are selected by badgers as urination sites, and this direct contamination of pasture enhances the disease risk to cattle.

Although there is a correlation between areas subjected to badger control operations and particular habitat characteristics, the amounts of these habitat features present in an area do not correlate with badger density. This suggests that badger density (in terms of the number of social groups per square kilometre) may not be a key variable identifying problem areas (cf. Wilesmith 1983). Variations in social group size may be a confounding factor, but the limited amount of data available suggests that there is no relation between badger group size and the prevalence of bovine tuberculosis in badgers (Cheeseman *et al.* 1988).

Although at present there is no evidence of a relation between badger group size and the prevalence of tuberculosis, it is still possible that there is an enhanced disease risk with larger badger social groups and that there is some relation between group size and habitat (Kruuk 1978; Kruuk & Parish 1982; Hofer 1988), but data on any such relations are scant. Indeed Cheeseman *et al.* (1987) have shown that badger social group size exhibits large variations from year to year, and can undergo significant long-term changes in the absence of any habitat changes.

To improve our understanding of the risk of disease transmission, more information is needed on the relations between badger group size and habitat, and on the different types of linear features and the pattern of crossing-point urinations in different land classes in the problem areas of the southwest. This should help determine why linear habitat features do not appear to be important in all the land classes examined. Other factors, particularly farm management practices such as grazing régimes and stocking densities, and seasonal and annual changes in the prevalence and incidence of the disease in badger populations, will undoubtedly also play a role in the transmission process and may also be correlated with features such as landscape heterogeneity. These possibilities all need to be examined in detail. However, the preliminary analyses presented here are potentially of great importance in helping understand why bovine tuberculosis in cattle remains localized in particular areas. We present the hypothesis that the urinatory behaviour of badgers on crossing-point runs, as determined by the type and density of linear habitat features, is a significant causative factor behind the correlation between the risk of disease transmission and landscape type in parts of southwest England.

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